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FATAL HERPES SIMPLEX ENCEPHALITIS IN MAN *

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For many years there has been considerable doubt as to the etiologic relationship between herpes simplex virus and human encephalitis.[1] The confusion has been due mainly to the fact that the virus has been isolated from the brain or spinal fluid of patients with such unrelated diseases as syphilis of the central nervous system about as frequently as it has been recovered from the brain or spinal fluid of patients with encephalitis lethargica.[1-4] Indeed, herpes virus has been isolated from the spinal fluid of apparently normal persons.[1] Such findings are explained on the basis that a large proportion of the population is believed to carry constantly the herpes virus which may be widely disseminated in the body tissues without causing evident disease.[1,5,6] For these reasons, herpes may be encountered frequently as a contaminant, and its isolation even from cases of encephalitis would appear to be of doubtful significance in most instances.

Recently, however, two cases have been described which seem to present adequate criteria for establishing the causal relationship between herpes and the meningo-encephalitic manifestations encountered. The first of these was reported by Smith, Lennette and Reames[7] who isolated herpes virus from the brain of an infant whose encephalitic lesions were unusual in that intranuclear inclusions of the herpetic type were present in many cells. Their presence in the brain of this patient may be considered as evidence of reaction of the host to the virus isolated from his cerebrum. Several other cases have been reported[8-10] in which such inclusions were described, but their proper evaluation is not possible as the causative agent was never determined.

The second case was that of Armstrong[11] who isolated herpes virus from the spinal fluid of an adolescent male with aseptic meningitis. He demonstrated the etiologic importance of this agent by means of serologic studies. Blood taken from the patient during the acute illness

* Received for publication, July 12, 1943.

had little or no capacity to neutralize a known strain of herpes virus, but that taken during convalescence contained neutralizing substances.

The present report describes the clinical and pathological findings in a fatal case of encephalitis with acidophilic intranuclear inclusions in cells of the brain substance. In addition, the results of studies which led to the isolation of an infectious agent from the brain and its identification as a strain of herpes simplex virus are given.

Report of Case *

History. M. B., a 25-year-old private in the United States Army, was admitted to the Station Hospital at Camp Claiborne, Louisiana, on July 27, 1942. He was unable to give a history. It was learned that he had complained of severe headache for 3 days prior to admission, and had become slow and incoherent of speech. There had been no recent vaccination and no history of herpetic eruption.

Physical Examination. Temperature, 103.8° F.; pulse, 108; respirations, 26; blood pressure, 142/88. The skin was hot and dry. There were no herpetic lesions. The lips and nail beds were slightly cyanotic. The throat was moderately injected. Neurologic examination revealed a conscious, apathetic patient whose answers were unrelated to questions asked. He seemed to know what objects were, but was unable to name them. The left pupil was slightly larger than the right; both reacted well to light and in accommodation. Extra-ocular movements were done well. The optic disks were of good color and showed no papilledema. No impairment of sensation could be detected. There was no paralysis or local motor weakness. A slight increase in tone of the right upper and right lower extremities was present. Deep tendon reflexes were equal and somewhat hypo-active. The plantar responses were normal. The neck was stiff and Kernig's sign was positive bilaterally. The remainder of the physical examination was negative.

Laboratory Findings. Blood. July 27: thick drop preparation was negative for malaria; red blood cells, 4,900,000 per cmm.; white blood cells, 16,200 per cmm.; polymorphonuclear leukocytes, 69 per cent.; lymphocytes, 21 per cent. July 30: white blood cells, 16,000 per cmm.; polymorphonuclear leukocytes, 92 per cent; lymphocytes, 8 per cent. Culture taken on July 27 showed no growth after 5 days.

Urine. July 27: negative.

Spinal Fluid. July 27: clear; pressure, 180 mm. of water; cells, 175 per cmm., all lymphocytes; organisms, none in smear; culture, no growth; sugar, normal reduction; globulin, 1 plus Pandy reaction; total protein, 44.4 mg. per cent; Wassermann reaction, negative. July 30: fluid flocculent in appearance; pressure, not increased; cell count, 323 per cmm. with 97 per cent lymphocytes; organisms, none in smear; culture, no growth; sugar, normal reduction; globulin, 2 plus Pandy reaction; total protein, 50 mg. per cent.

Roentgenographic Findings. Bedside examinations of the chest on July 28, 29 and 30 showed no evidence of pneumonia.

Clinical Course in Hospital. Following the first lumbar puncture, the patient became slightly more rational than on admission. The next day his temperature reached 105° F. and he continued to be restless, complained of headache, became more drowsy and then completely delirious. During his hospital stay of 5 days, the temperature remained elevated between 103° and 105° F., with a pulse rate of around 110. No further neurologic abnormalities were noted. On July 30, he

* Accession number 85691, Army Institute of Pathology of the Army Medical Museum, Washington, D. C.

became more comatose and developed râles and rhonchi in his lungs. Hiccoughing became quite troublesome. He was given intravenous fluids, and, because of the increasing signs of pneumonia, 5 gm. of sodium sulfathiazole intravenously. However, he became progressively weaker, lapsed into coma, and died on August 1, 5 days after admission. The clinical diagnosis was equine encephalomyelitis.

Autopsy Findings

The autopsy was performed 3 hours post-mortem. Gross pathologic changes were limited to the chest and brain. There was bilateral hydrothorax, with about 500 cc. of clear straw-colored fluid in each pleural cavity. Evidences of bronchopneumonia were present in the dependent portions of the lungs.

The brain was removed without difficulty. The dura was moderately tense. A slight excess of cerebrospinal fluid was noted. The gyri were moderately flattened and the hemispheres were symmetrical. The meninges were smooth and uniform throughout. The vessels over the surface were slightly congested, and there was moderate edema of the parenchyma. The brain weighed 1,350 gm. Along the inferior margin of the left temporal lobe was an area of softening about 4 cm. in diameter. This area was studded with small hemorrhages which on section were seen to extend into the parenchyma.

Microscopic Examination. Significant pathologic lesions observed on microscopic examination were limited to the lungs and the central nervous system. An early confluent bronchopneumonia with an exudate consisting principally of polymorphonuclear leukocytes was found.

Intense vascular congestion was uniformly present in the central nervous system, with many areas of small ecchymoses and petechiae in both the brain substance and subarachnoid area. A moderately severe focal meningitis, with infiltrations of lymphocytes and large mononuclear cells generally occurring about vessels, was found over the cerebral hemispheres and the cerebellum and about the spinal cord.

The most striking lesions in the brain substance were found in the cortex and subcortical white matter, particularly in the vicinity of the area of softening in the left temporal lobe. Perivascular cuffs composed of lymphocytes in the Virchow-Robin spaces were present throughout this region and were occasionally encountered in the midbrain and pons. The endothelial cells lining such vessels were frequently swollen. Proliferated glial cells were numerous in the tissue about the cuffed vessels. Within the area of encephalomalacia in the left temporal region there were numerous small hemorrhages, as well as foci of spongy necrosis and large collections of "Gitterzellen" among which a moderate number of polymorphonuclear leukocytes were visible. Lamination of the cortex was difficult to make out even in areas distant from

the severely affected portion of the temporal lobe. The ischemic necrosis of many scattered nerve cells together with an increase in the number of glial cells was responsible for the disruption of the normal architecture. Necrosis of nerve cells occurred rarely or not at all in the midbrain, pons, cerebellum and cord.

Intranuclear inclusions of the herpetic type were present in profusion in certain portions of the cerebrum. These appeared as bright pink, homogeneous or granular structures occupying most of the space within the nuclei of cells in sections stained with phloxine and methylene blue or by Giemsa's technic. Occasionally the characteristic picture of an A type intranuclear inclusion was seen, *i.e.*, a large pink body surrounded by a clear zone in which the blue nucleolus lies close to the dark nuclear membrane which is thickened because of margination of chromatin. Most of the acidophilic inclusions were found in glial cells but a few nerve cells were similarly affected. Inclusions were encountered most frequently in or near those portions of the pyramidal layers where necrosis of cells had occurred. They were seen also in cells deep in the brain tissue in the areas of glial infiltration which surrounded cuffed vessels. Intranuclear inclusion bodies were not observed in sections from the midbrain, cerebellum, pons, or cervical cord.

Isolation of the Virus

Attempts were made to isolate virus from spinal fluid and blood drawn during life, and from spinal fluid and brain material removed at autopsy.

Two days before death, specimens of blood and spinal fluid were shipped frozen to the Army Medical School. Guinea-pigs were inoculated intracerebrally and intraperitoneally with a mixture of the two fluids, and, while transient fever resulted in two animals, no transmissible disease was obtained.

The spinal fluid removed immediately after death proved to be bacteriologically sterile. At the Station Hospital, it was injected intracerebrally into six mice, four of which died after about 3 days. Cultures of the brains were all negative. A suspension was made from two of these, and injected into two more mice. One failed to show signs of illness, but the other succumbed on the third day. Sections of the mouse brains were not interpretable because of post-mortem change. The agent was not maintained because of lack of facilities at Camp Claiborne. However, in the light of later findings, it appears that the virus was present in the spinal fluid obtained at the autopsy.

At the time of autopsy, sections were removed with sterile precautions from the temporal lobe, motor cortex, midbrain, pons, cerebellum and cervical cord. These were placed in sterile buffered glycerin and

sent to the Division of Virus and Rickettsial Diseases at the Army Medical School, where they were received 4 days later. Four separate attempts were made before isolation of the virus was accomplished. Ten per cent suspensions of cerebral tissue were injected intracerebrally and intraperitoneally into guinea-pigs. A febrile response which began in about 5 to 7 days and lasted for several days was the only evidence of disease induced in these animals. Although response of this type was elicited on several occasions, the disease was not transmissible from the original to passage animals.

Approximately 6 weeks after receipt of the glycerinated material, during which interval it had been stored at 5° C., fresh suspensions were prepared and injected intracerebrally into mice and into young adult hamsters.

Three mice received intracerebral inoculations of 0.03 cc. of a 10 per cent suspension of the brain in serum-saline solution. One mouse showed paralysis and convulsions on the eighth day. It was sacrificed and its brain cultured and passed in a like manner to additional mice. These animals died on the fourth day.

The virus* was then maintained by successive mouse passages. The other two original mice failed to develop any evidence of disease. They were later subjected to intracerebral inoculation of fifth-passage mouse brain and found to be immune.

Two young Syrian hamsters were inoculated intracerebrally with 0.05 cc. of the same suspension of original brain material. One became ill on the sixth day. It was sacrificed and its brain passed to two more hamsters, and to mice. The mice died with typical clinical findings for the virus strain, confirming its presence. The other hamster failed to develop evidence of disease. However, its serum was later found to afford a significant degree of protection in neutralization tests against the virus.

Of the second-passage hamsters, one became ill. Though its brain was passed to other hamsters, they failed to develop clinical evidence of disease and this substrain was considered lost.

Manifestations of the Virus in Laboratory Animals

Mice. The virus was carried through 33 consecutive passages in mice. In these animals the 50 per cent lethal end-point became stabilized at 10^{-3} to 10^{-4}. Following intracerebral inoculation, the disease usually manifested itself first as a marked hyperactivity to mechanical stimuli. This occurred as early as 24 hours after inoculation, but more commonly after 2 to 3 days. Once this sign appeared, the course was

* The virus isolated from this patient is designated as the "M. B. virus" throughout the text.

generally a rapid one. The animals developed a roughened fur, their gait became spastic, they lost their normal activity, and ate and drank poorly. Death usually occurred following a tetanic convulsive seizure, the front and hind legs extended backwards.

A somewhat different picture was observed in those mice which died following intraperitoneal inoculation with larger amounts of virus. The course was more prolonged, death occurring after a week or more. The animals appeared sluggish, their fur became roughened, but the hyperactivity and convulsive seizures did not usually occur. Death occurred suddenly and rather unexpectedly when compared to the course of mice inoculated intracerebrally. That the brains of such mice contained the virus could readily be demonstrated by intracerebral passage into other mice, which followed the usual clinical pattern.

Hamsters. Three young hamsters (3 to 4 weeks old) were inoculated intracerebrally with 0.05 cc. of a 1:10 dilution of brain from the 17th mouse passage. All were ill on the second day. One was sacrificed for further passage, the second died on the fifth day, while the third recovered. The second-passage hamsters also became ill on the second or third day, as did those of the third passage. Virus from a third-passage hamster brain was titrated intracerebrally in mice and found to kill in dilutions up to and including 1:10,000. Illness in hamsters was manifested principally by hyperactivity, followed by sluggishness, muscular incoordination, tremors, and in some instances paralysis and death.

Intraperitoneal inoculation usually produced no clinical disease in hamsters. However, one of the third-passage hamsters used in the original attempt at isolation of the virus, and which had shown no clinical signs of disease, was inoculated intraperitoneally 24 days later. It received 0.2 cc. of a 10 per cent suspension of brain from the fourth mouse passage. Nine days later, this hamster developed paralysis of the hind legs. It was sacrificed and brain material in 1:10 and 1:100 dilutions was injected intracerebrally in mice. These died in the expected manner, indicating the presence of the virus.

Guinea-pigs. Two guinea-pigs received 0.1 cc. intracerebrally of a 1:10 suspension of mouse brain from the 10th serial passage. Both animals had fever from the fourth day. One showed weight loss and kyphosis. It underwent generalized clonic convulsions on the seventh day. The other animal was sacrificed on the sixth day. Its brain was passed intracerebrally to additional guinea-pigs and mice. These guinea-pigs had a mild elevation of temperature from the fourth to sixth days, then recovered. The mice, however, died from 3 to 5 days following inoculation.

Intracutaneous and foot-pad inoculations were also done in guinea-pigs. Red papular lesions appeared at the sites of intracutaneous inoculation on the second day, subsiding in 2 to 3 days. One such lesion developed a vesicle before resolving. The inoculated foot pads became inflamed and swollen on the second day, the reaction gradually subsiding in the course of a few days. Lesions of the skin and foot pads were surgically removed, ground, suspended in serum-saline solution and similar inoculations were made in other guinea-pigs with comparable results. The presence of virus in these lesions was verified by mouse inoculation. There were no deaths among guinea-pigs inoculated through peripheral routes.

Rabbits. Three rabbits were inoculated with a suspension of potent mouse-brain material on the scratched corneal surface. One showed clouding of the cornea on the second day. This progressed to a marked keratitis and conjunctivitis. The animal also developed signs of encephalitis and was sacrificed on the fifth day. Brain was titrated in mice, only a small amount of virus being present. The second rabbit developed marked conjunctivitis, keratitis and iritis. The process gradually resolved, and the animal recovered without developing encephalitis. No corneal or encephalitic disease was observed in the third rabbit.

Two rabbits received intracutaneous and subcutaneous inoculations of infectious mouse brain without the production of significant lesions. Negative results were obtained also with intracerebral inoculations of two rabbits.

Rhesus Monkey. An old monkey, which had been used for other studies, was inoculated with a 10 per cent suspension of brain material of known infectivity from the 20th mouse passage. It received 0.4 cc. intracerebrally, 0.4 cc. subcutaneously, as well as scratch inoculations of the skin and cornea. No fever or lesions resulted.

Chorioallantoic Membrane of Developing Chick Embryo. The virus was carried through five successive transfers on the chorioallantois of developing chick embryos. Opaque central plaque lesions were seen on the second and third day following inoculation. These lesions were surrounded by smaller pock-like foci. Grossly and microscopically the lesions resembled, in general, those described for herpes simplex virus.[12, 13]

Pathology of Brains of Experimental Animals

Histopathologic studies were employed as an ancillary aid in the identification of the M. B. agent. Selected tissues from infected rabbits, mice, guinea-pigs and hamsters, and the chorioallantoic mem-

branes of embryonated eggs were fixed in acetic Zenker's solution, and paraffin sections were prepared and stained by a modification of the Romanowsky technic. The lesions were similar to those described by others in studies of experimental herpes (van Rooyen and Rhodes,[1] Smith, Lennette and Reames[7]). Particular attention was paid to the changes in the brains of mice since these animals were used most extensively, and of hamsters for their response to herpes virus has not been previously described.

It seems worth while to emphasize the characteristic microscopic findings in brains of mice that died 2 to 3 days after intracerebral inoculation with a well adapted neurotropic strain of herpes virus. Infiltrative phenomena, *i.e.*, perivascular collections of lymphocytes in the brain substance and in the meninges, had not yet appeared. Moreover, examination under low power showed little except focal areas in which nerve cells stained poorly. Closer study of such areas revealed the presence of necrobiotic changes which ultimately reach necrosis. In the less severely affected cells the classical intranuclear inclusions of herpes could be found. Mice that died following a more prolonged incubation period developed the meningo-encephalitis usually described; however, intranuclear inclusions were difficult to find in such brains. When present, they were usually encountered about the perimeter of fresh areas of necrosis.

The primary cellular changes induced by various viral infections range from those almost entirely degenerative to those almost entirely proliferative; while inflammatory reactions, as evidenced by infiltrative phenomena, are secondary lesions.[14] The early lesion of herpes simplex in the mouse brain is essentially degenerative in character. Histopathologic changes encountered in the brains of hamsters, rabbits and guinea-pigs infected with the mouse-adapted strain of M. B. virus were similar to those observed in mice; intranuclear inclusions of the herpetic type were found in each species. In addition, a few intranuclear bodies were observed in the proliferating ectodermal cells in the pock-like lesions on the chorioallantoic membranes of eggs inoculated with the M. B. virus.

Identification of the M. B. Virus

The cardinal feature of the microscopic pathology in both the human and the animal brain was the presence of acidophilic intranuclear inclusion bodies in the nerve cells. Such inclusions are especially associated with infection with herpes simplex, B virus and pseudorabies.[1] Separation of M. B. virus from B virus and pseudorabies was suggested by the differences in host range, routes of infectivity, and clinical

course in experimental animals. For example, B virus produces encephalitis irregularly in mice inoculated intracerebrally, and Sabin[15] was unsuccessful in several attempts at mouse passage with brains of mice sacrificed at the height of the disease. In addition his results from inoculations of rabbits with B virus were more pronounced than those we obtained with the M. B. strain. Pseudorabies was considered improbable since none of the rabbits inoculated with the M. B. virus exhibited the characteristic "mad-itch" syndrome ascribed to it.[1] It was apparent, therefore, that we were most likely dealing with a strain of herpes simplex virus. The following studies were undertaken to explore this possibility.

Experiments Designed to Establish the Identity of the M. B. Virus. The methods used to identify the M. B. strain of virus included the observation of the R. T. strain* of herpes simplex in parallel animal experiments, as well as cross-immunity and cross-neutralization tests.

The routes of infectivity, period of incubation, clinical course, type of death and pathologic findings for both strains of virus were essentially identical in mice, hamsters, guinea-pigs, rabbits, and the chorioallantoic membranes of developing chick embryos.

Cross-Immunity Tests in Mice. Mice were immunized with some difficulty to our strain (M. B.) of virus and to the known strain (R. T.) of herpes simplex. Immunization was accomplished by repeated injections of the virus in graduated doses. Two or three injections were made subcutaneously and then intraperitoneally, at intervals of a few days to a week apart. Finally, an intracerebral inoculation with the homologous virus was made to test for immunity.

In the course of these experiments, 11 mice were immunized with M. B. virus (Table I). Ten of these survived intracerebral inoculation with the R. T. virus, 1 animal dying 14 days after inoculation, probably from intercurrent infection. All normal control mice died after undergoing the characteristic course.

In the reverse cross, five mice, which had been immunized with R. T. virus, were tested. All survived intracerebral inoculation of a 1:10 dilution of the M. B. virus. The control animals all died.

Cross-Neutralization Tests in Mice. The preparation of potent neutralizing sera for herpes simplex virus is difficult.[7, 11] In general, our efforts with the M. B. virus and the R. T. strain proved to be no exception. Guinea-pigs, rabbits, hamsters and one monkey (receiving M. B. virus) were repeatedly inoculated subcutaneously and intraperitoneally with suspensions of infected mouse brain. Three to five

* The R. T. strain of herpes simplex virus was kindly furnished us by Dr. Margaret M. Smith.

injections were given each animal at intervals of a few days to 2 weeks, with a final intracerebral inoculation to check immunity to the virus strain used. After a period of 2 weeks or more from the last injection, the animals were bled.

Neutralization tests were carried out in the following manner: suspensions of fresh* infected mouse brain in sterile serum-saline solution were made up in serial ten-fold dilutions. Equal amounts of the virus dilution and of serum were thoroughly mixed, incubated in the water-bath at 37.5° C. for 1 hour, then inoculated intracerebrally into mice.

TABLE I

Cross-Immunity Tests in Mice: M. B. Virus and Known Herpes Simplex Virus (R. T. Strain)

Experiment		Immunized with	Tested with*	Deaths among tested mice	
				Immunized	Normal
I	A	M. B. virus	R. T. virus	0, 0, 0, 14†	7, 7, 7, 10
	B	M. B. virus	R. T. virus	0, 0	3, 3, 3, 3, 3, 4
	C	M. B. virus	R. T. virus	0, 0	2, 3, 3, 3, 3, 3
	D	M. B. virus	R. T. virus	0	4, 4, 4, 4, 4, 4
	E	M. B. virus	R. T. virus	0, 0	4, 5, 5, 6, 7, 7
II	A	R. T. virus	M. B. virus	0	2, 2, 3, 3, 3, 3
	B	R. T. virus	M. B. virus	0, 0, 0, 0	3, 3, 3, 4, 4

* Test dose consisted of an intracerebral inoculation (0.03 cc.) of a 10^{-1} dilution of fresh infected mouse brain. Such a suspension of virus contained 10^{3} to 10^{4} MLD.

† This mouse probably died of intercurrent infection; the numeral indicates day of death of mouse following test inoculation; 0 indicates mouse survived.

Daily observations of the mice were continued for 18 days following inoculation.

Though not so striking as desirable, the results supported the cross-immunity findings, which indicated a close similarity of the two strains of virus. In general, the immune sera protected against three to five times as much virus of both strains as did the control sera. In Table II are given the results obtained in two experiments with guinea-pig sera. Comparable results were obtained in tests with monkey, hamster and rabbit sera. In some instances, sera gave better protection against the homologous virus strain than against the heterologous strain, while in others the reverse was noted.

SUMMARY

A virus was isolated from the brain of a fatal case of acute encephalitis. Acidophilic intranuclear inclusion bodies were present in the

*Fresh mouse brain was used since it was found that the potency of the two virus strains rapidly declined when stored at −70° C. Such fresh material repeatedly yielded a titer of 10^{-3} or better when tested intracerebrally in mice.

TABLE II

Cross-Neutralization Tests: M. B. Virus and Known Herpes Simplex (R. T. Strain) with Guinea-Pig Sera

Virus strain	Virus dilution	Experiment I: Normal serum	Experiment I: M. B. immune* pool 811–812	Experiment I: R. T. immune pool 806–808	Experiment II: Normal serum	Experiment II: M. B. immune 455	Experiment II: R. T. immune 341
M. B.	10^{-1}	.....	.....	.....	4, 4, 5, 6, 7, 7†	0, 0, 0, 0	4, 4, 4, 5
M. B.	10^{-2}	.....	6, 6, 6, 7	5, 5, 6, 7	6, 6, 6, 9, 9, 18	10, 0, 0, 0	8, 10, 10, 11
M. B.	10^{-3}	4, 4, 5, 6	7, 7, 8, 0	6, 8, 11, 0	7, 14, 15, 0, 0, 0	0, 0, 0, 0	7, 0, 0, 0
M. B.	10^{-4}	5, 7, 8, 10	0, 0, 0, 0	15, 0, 0, 0	10, 0, 0, 0, 0	.....	.....
M. B.	10^{-5}	8, 8, 0, 0	.....	.....	.....	.....	.....
M. B.	10^{-6}	13, 0, 0, 0	.....	.....	.....	.....	.....
Virus titer		$10^{-5.2}$	$10^{-3.3}$	$10^{-3.5}$	$10^{-3.2}$	$<10^{-1.0}$	$10^{-2.7}$
R. T.	10^{-1}	.....	.....	.....	3, 3, 4, 4, 4, 4	6, 7, 9, 9	4, 5, 5, 5
R. T.	10^{-2}	.....	0, 0, 0, 0	7, 7, 8, 11	4, 5, 5, 5, 7, 7	11, 13, 0, 0	5, 6, 6, 7
R. T.	10^{-3}	6, 11, 0, 0	10, 0, 0, 0	0, 0, 0, 0	5, 5, 6, 7, 7, 8	9, 0, 0, 0,	10, 11, 12, 12
R. T.	10^{-4}	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	8, 10, 10, 13, 0, 0	.....	.....
R. T.	10^{-5}	0, 0, 0, 0	.....	.....	.....	.....	.....
R. T.	10^{-6}	0, 0, 0, 0	.....	.....	.....	.....	.....
Virus titer		$10^{-3.0}$	$<10^{-2.0}$	$10^{-2.5}$	$10^{-4.3}$	$10^{-2.2}$	At least $10^{-3.5}$

* Guinea-pigs received repeated subcutaneous and intraperitoneal injections of the virus strain and an intracerebral challenge with the same virus before being considered immune.

† Numeral indicates day of death of mouse following intracerebral inoculation of serum-virus mixture; 0 indicates mouse survived at least 18 days.

affected brain cells of the patient, and were also produced in experimental animals. The presence of these bodies suggested that the virus agent belonged to the group which includes herpes simplex, B virus and pseudorabies. Elimination of B virus and pseudorabies was based on the type of response elicited in laboratory animals. Identification of the M. B. virus as herpes simplex was made through cross-immunity tests and cross-neutralization tests with a known strain (R. T.) of herpes virus.

This is the second case of fatal herpes simplex encephalitis associated with acidophilic intranuclear inclusions from which the virus has been isolated and its etiologic relationship established.

We wish to thank Capt. H. Hamilton, Sn. C., who carried out the chorioallantoic studies, and Lt. K. Wertman, Sn. C., who assisted with the earlier animal work. Many members of the Virus Division of the Army Medical School contributed valuable assistance in the course of these studies.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 84

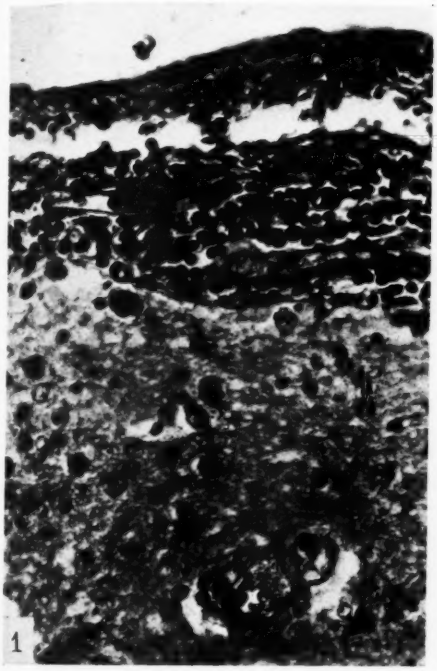
Pathologic changes in the brain of a patient with herpes encephalitis. The sections were stained with hematoxylin and eosin.

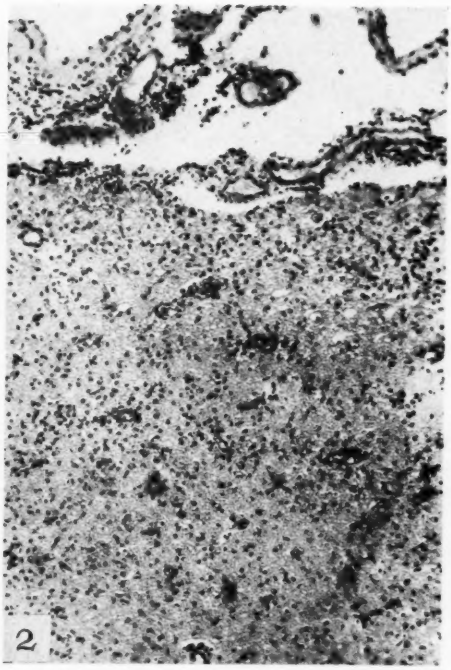
FIG. 1. The leptomeninges contain lymphocytes, and the underlying cortex is infiltrated with microglia and astrocytes. × 370.

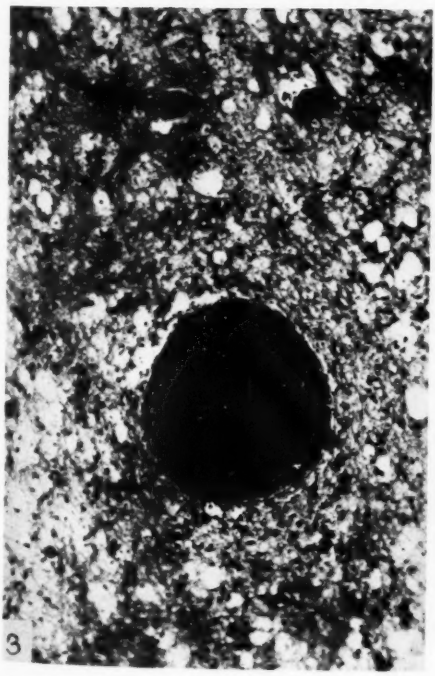
FIG. 2. Leptomeninges and cortex. The ganglion cells are in a state of degeneration. The cortex contains numerous microglia and a few astrocytes. × 90.

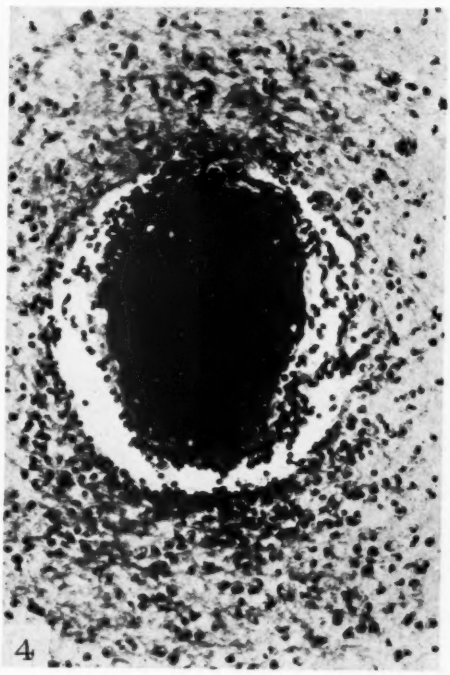
FIG. 3. Subcortical white matter. The tissue is in part necrotic. Microglial cells are accumulated in the extra-adventitial tissue. × 115.

FIG. 4. Subcortical white matter. The Virchow-Robin space surrounding the vessel contains numerous lymphocytes. The extra-adventitial tissue is crowded with Gitterzellen. × 115.









Zarafonetis, Smadel, Adams, and Haymaker

Herpes Simplex Encephalitis in Man

PLATE 85

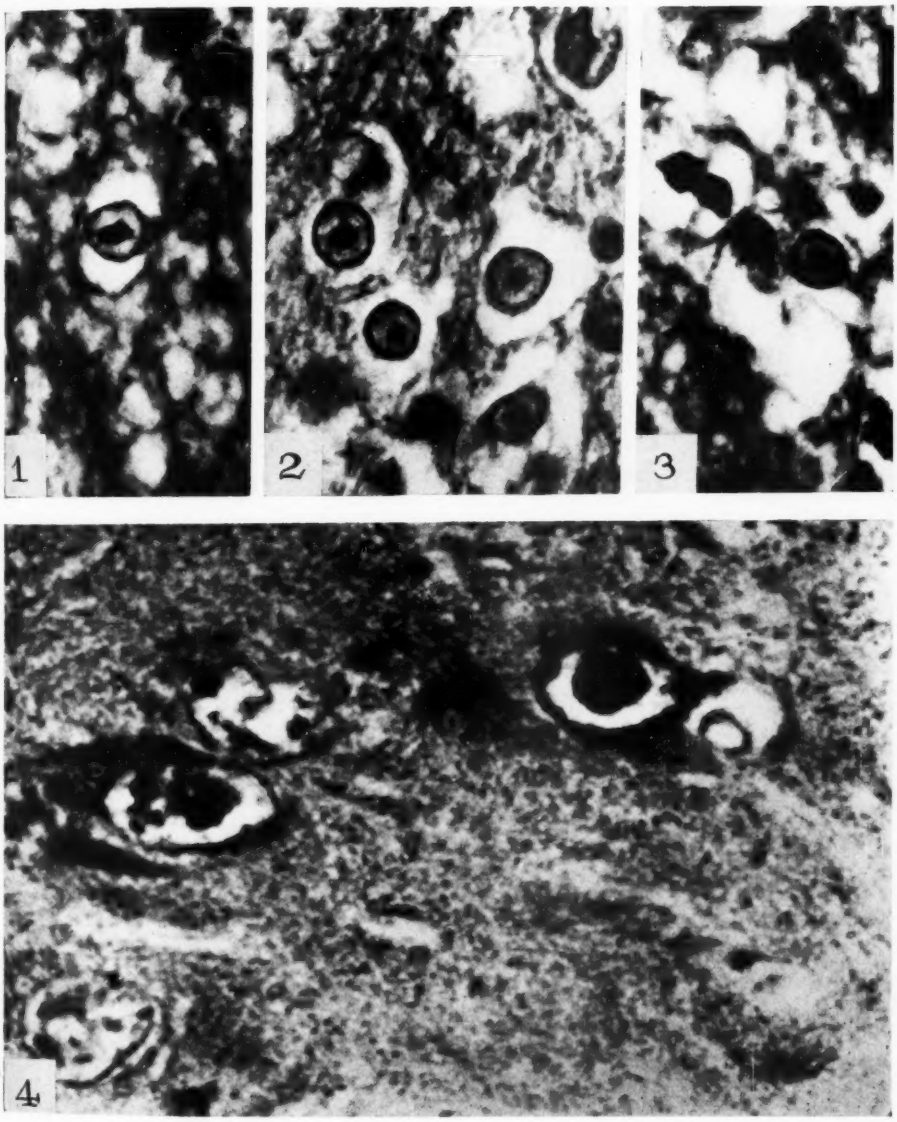
Intranuclear herpetic inclusions in human and murine brains. The sections are stained with phloxine and methylene blue.

FIG. 1. Intranuclear inclusions in human brain. × 1075.

FIG. 2. Intranuclear inclusions in human brain. × 1075.

FIG. 3. Intranuclear inclusions in human brain. There is margination of the nuclear chromatin. × 1075.

FIG. 4. Intranuclear inclusions in brain of mouse. One of the inclusion bodies has a homogenous appearance; the other is granular. × 2150.



Zarafonetis, Smadel, Adams, and Haymaker Herpes Simplex Encephalitis in Man

INFECTION OF HUMAN SKIN, GRAFTED ON THE CHORIOALLANTOIS OF CHICK EMBRYOS, WITH THE VIRUS OF HERPES ZOSTER *

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Although herpes zoster has not been undoubtedly transmitted to experimental animals there is good evidence that its causative agent has been successfully inoculated into human beings and that it is a virus. The first to accomplish human inoculation was Kundratitz[1] in 1925. In his first experiments three children were inoculated after scarification on the upper arm, with clear contents of zoster vesicles. In two of these, after 11 days, there appeared in the area of inoculation several clear vesicles with a slight reddening of the periphery. The appearance corresponded quite completely with inoculatory vesicles of varicella first induced locally in man by Kling[2] in 1913. The vesicles healed during 2 or 3 days and became dry.

Following these first positive results Kundratitz inoculated material from nine typical cases of thoracic zoster into human skin. Material from four of these resulted in typical vesiculation locally in the skin of several children. The reaction appeared 9 to 12 days after inoculation.[1]

In 1921, Lipschütz[3] described acidophilic intranuclear inclusions in the cutaneous lesions of zoster which he considered to be specific. His observations have been repeatedly confirmed and now the presence of acidophilic intranuclear inclusions is regarded as the most specific characteristic of the zoster lesion, although the inclusions are indistinguishable from those first described by Tyzzer[4] in cutaneous lesions of varicella. Their presence is taken to signify the effect of a virus. Kundratitz removed some of his experimental lesions and submitted them for histological study to Lipschütz who demonstrated in them the characteristic inclusions. The successful inoculations were in children under 5 years of age, some of them nurslings; those in older children were negative.

The etiological agent of herpes zoster has never been successfully inoculated into any animal in series other than man, although Lipschütz[3] and Marinesco and Draganesco[5] reported the presence of acidophilic intranuclear inclusions in the corneal epithelium of rabbits after inoculation with vesicular contents. Others, including Cole and Kuttner,[6] who reviewed the subject, failed to confirm these results.

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Further light on the nature of zoster virus and its relation to other viruses, especially that of chicken-pox which some investigators believe to be identical, awaits the discovery of a susceptible experimental animal other than man, or the development of a technic by means of which these important problems can be studied.

In attempts to devise a method of this sort, Goodpasture, Douglas and Anderson,[7] in 1938, succeeded in grafting human skin onto the chorioallantois of chick embryos so that it would survive for several days. Such human skin grafts were successfully inoculated with the viruses of herpes simplex, variola and vaccinia, but all of our attempts to infect the grafts with the virus of chicken-pox failed and in only one set of experiments, out of several, in which human skin grafts were inoculated with the clear fluid from zoster vesicles, did we succeed in inducing specific lesions of this disease. Inoculations of grafts of human fetal membranes (amnion, chorion) with material from vesicles of varicella and zoster were all unsuccessful.[8]

Although we succeeded only once after several attempts in infecting with zoster virus human skin grafted on the chick's chorioallantois, we decided to describe our positive results in the hope that others might have more favorable opportunity to use the method with success. The problem of obtaining suitable skin for grafting and at the same time to be in possession of uncontaminated fluid from zoster vesicles we found to be a difficult one, especially because our facilities permitted us to obtain only minimal quantities of fluid from occasional cases of zoster.

Human Skin Grafts

The technic for grafting human skin onto the chorioallantois of chick embryos we have previously published[7] in detail and it need not be repeated here. We will therefore describe only the materials and conditions of our successful experiments with zoster, the circumstances of which possess some points of interest.

Source of Human Skin for Grafts. The patient was a white woman, 57 years of age, upon whom a radical mastectomy was performed for scirrhous carcinoma of the right breast. The defect was closed with a single Thiersch graft taken from the right thigh. Pieces of this skin were used to graft on the chorioallantois of 12 9-day-old chick embryos.

It is of interest that the donor gave a history of an attack of shingles in the right thoracic region 8 months previous to her operation.

Source of Herpes Zoster Virus. Clear, vesicular fluid was removed from a band of eruption extending across the right side of the back and into the axilla of a child, 2½ years old. This child had experienced a typical attack of chicken-pox 1 year before admission for zoster.

The clinical description of the zoster eruption was as follows: Starting at the midline in the back at about the second thoracic vertebra there was a band of splotchy erythema about 2 cm. in width extending anteriorly to the midsternal line. There was one small erythematous patch, 1.5 cm. in diameter, high in the axilla upon which were multiple small vesicles not larger than 1 mm. in diameter. Several more patches of blisters were situated anteriorly in which the vesicles were much larger. It was from the latter, especially, that material was obtained for inoculation of the grafts. There was no pustulation.

Inoculation of the Grafts

The skin was prepared by the surgical routine for grafting consisting of scrubbing with soap and water and the application of tincture of iodine which was followed by alcohol. After removal, pieces of skin were delivered to us in moist sterile gauze. These pieces were spread out on sterile cork blocks with the epidermis upward. They were cut into 1 cm. squares. Upon the epidermis of each was placed a drop of undiluted vesicular fluid from the syringe in which it was aspirated. With the syringe needle (size 27) multiple punctures were made through the fluid into the surface of the skin. An attempt was made to keep all of the fluid confined to the central portion of the graft. After puncturing, the graft was left for 1 hour on the cork, moistened along the edges with sterile saline solution and lightly covered with moist gauze. This was done in order to allow the vesicular fluid to be absorbed by the skin while the underlying corium was kept in a moist state.

Each graft was then placed upon the chorioallantois of a 9-day-old chick embryo in the manner we have previously described. The grafts adhered successfully and the eggs were incubated for 4, 6, 7 and 8 days, at which intervals living eggs were opened and the grafts with the adherent chorioallantois were removed and fixed in Zenker's solution for histological study. Suggestive but indefinite pin-point lesions were seen grossly on some of the grafts.

After paraffin embedding, sections were stained with hematoxylin and eosin.

Description of the Lesions

Five grafts removed from this series of embryos showed microscopical lesions which were interpreted as evidence of infection with the virus of herpes zoster. One of these was fixed 4 days after inoculation and grafting, 1 on the sixth day, 2 on the seventh day and 1 on the eighth day. Grafts made on the fourth and sixth day showed small foci in an early stage of development; the 8-day graft contained larger

and pustular foci, as many as three being present in a single section. That the foci of infection corresponded with points of puncture at the time of inoculation was indicated in some by the presence of a foreign body reaction beneath the center of the lesion in the chorion or underlying chorioallantois, where evidently a minute fragment of keratin was forced through.

The earliest lesions consisted of small groups of hyperplastic epithelial cells situated in or near the basal layer and free from inflammation. Both cytoplasm and nuclei were swollen and each of the latter contained within its clear nucleoplasm a large, compact acidophilic body. The nuclear chromatin and often the nucleolus were situated peripherally next to the thin nuclear membrane. In older lesions the number of cells involved was much greater, the individual cells were enormously swollen, the enlargement involving both cytoplasm and nucleus. In the center of such a focus the epithelial cells were separated one from another by inflammatory fluid and cellular exudate consisting largely of chick leukocytes. Cellular exudate was present in the corium and underlying chorioallantois, especially abundant about blood vessels. Lying here and there in the purulent exudate of the infected epithelial layer were occasional multinucleated epithelial giant cells, each nucleus or nuclear lobule of which contained an acidophilic inclusion.

The lateral margins of these larger foci showed an extension of the infection to adjacent groups of epithelial cells. These cells were swollen, pushed apart by fluid and contained intranuclear acidophilic inclusions, but there was no cellular exudate.

Few of the involved epithelial cells, even in the most advanced lesions, were evidently necrotic; only rare ones stained so deeply with eosin as to indicate death. In none of the lesions had sufficient fluid accumulated to cause gross vesiculation.

In none of the specimens was there evidence of infection of chick cells as would be indicated by the presence of intranuclear inclusions. In spite of numerous attempts we have never been able to induce in any chick tissue lesions indicative of infection with material from herpes zoster. This is in contrast with the susceptibility of chick embryo tissues, including the chorioallantois, to the virus of herpes simplex.

Discussion

There is acceptable evidence that the characteristic lesion of both varicella and herpes zoster can be induced locally by direct inoculation of the respective vesicular fluid in scarified human skin.[1, 2] Our failures thus far with chickenpox, and similar numerous failures with zoster,

in attempts to infect grafts of human skin and fetal membranes, amnion and chorion, leave us without an adequate explanation. Although it might be assumed that the original grafts were immune to these viruses, we have demonstrated in other experiments with skin of chickens immune to fowl-pox and human skin immune to vaccinia that, when grafted onto the chorioallantois, the acquired immunity is lost and susceptibility returns.[9] In the successful experiments herein reported there was good reason to believe that the donor was immune to zoster inasmuch as she had experienced an attack of shingles only 1 year previously.

Reports of successful human transmission of cutaneous infections with the viruses of chicken-pox and of herpes zoster have indicated the first appearance of lesions in 8 to 11 days, whereas following inoculation with herpes simplex lesions appear in 2 to 4 days. In our successful experiments with zoster the first lesions without inflammation appeared in grafts of 4 days' duration, whereas with herpes simplex full-blown lesions occur in 2 to 3 days.

Most successful transmission experiments with zoster fluid have been in infants or children under 5 years of age, possibly because of lack of acquired immunity. In our experiment skin grafts were from an adult 57 years of age, and presumably these grafts lost their acquired immunity as a result of transplantation to the chick embryo. As is the case in infection of grafts of human amniotic epithelium with the virus of mare abortion, the successful inoculations of human skin with zoster fluid demonstrate that a natively susceptible tissue grafted on a naturally insusceptible host (chick embryo) does not thereby lose its susceptibility.[8]

In view of our negative results in several similar attempts to infect human grafts with zoster fluid the thought has occurred to us that the skin of some persons might be especially susceptible to this virus as indicated possibly by a previous attack of shingles in our donor. On the other hand, our successful experiment occurred in the only series in which both fresh zoster fluid and fresh skin were obtained and used on the same day; and this fact might have contributed in some way to the positive outcome.

In spite of several attempts we have not yet succeeded in infecting human skin or fetal membrane grafts with the virus of chicken-pox. If this technic were developed so that both varicella and herpes zoster could be uniformly induced in grafts of human tissue, there would be the possibility of using it to determine the relationship of these viruses to each other, and to discover some of their properties in the absence of a susceptible experimental host other than man.

Our experiments indicate that the epithelial cells of human skin may be immediately susceptible to the zoster virus and that an initial infection of nervous tissue is not essential to the development of a cutaneous eruption.

Notwithstanding the development of a local pustular lesion in the grafted skin, associated with swelling and disruption of cellular contacts and the appearance of multinucleated epithelial giant cells, the final criterion of infection with the zoster virus is the presence of intranuclear acidophilic inclusions indistinguishable from those in the lesions of chicken-pox, as first described by Tyzzer,[4] and those of zoster, as noted first by Lipschütz.[3]

Because of the fact that clinically and histologically the eruptive cutaneous lesions of chicken-pox and zoster may be indistinguishable, and the further fact that clinically chicken-pox may be associated with a zosteriform lesion and that zoster may be associated with a generalized nonneural cutaneous eruption, it is of great importance to use typical clinical cases of these diseases in experimental attempts to demonstrate and transmit a specific virus.

The lesions induced in human skin grafts inoculated with zoster fluid do not closely resemble those of herpes simplex in similar tissue. In the latter the inclusions become much larger, often filling the nucleus; and they acquire a basophilic staining reaction.[8] Simplex virus rapidly kills the cell, causing ulceration and an excessive purulent exudation.

Experimental zoster lesions in grafted human skin serve to throw some light on the pathogenesis of the zoster eruption, a subject which is still a matter of controversy. Only recently a review by Baird[10] so overemphasizes the participation of a neurovascular mechanism as to imply a denial of the local presence of a virus in the skin. No mention is made in that paper of cellular inclusions in the vesicles of zoster.

It is clear from our experiments that lesions composed of epithelial cells containing the zoster inclusions and later infiltrated with chick leukocytes can follow inoculation of human skin removed from its normal nervous and vascular connections. The failure of these lesions to develop completely as vesicles indicates the importance of neurovascular connections for the formation of the abundant serous exudate in the human eruption, but these relations to nerves and vessels do not determine the specific character of the lesions.

Conclusions

1. Grafts of human skin on the chorioallantois of chick embryos were successfully infected with the virus of herpes zoster.

2. The experimental lesions did not vesiculate grossly but became pustular, and otherwise resembled those in the natural disease, including the presence of intranuclear acidophilic inclusions, first described by Lipschütz,[8] in the affected epithelial cells.

3. The tissues of the chick embryo appear to be nonsusceptible to this virus.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

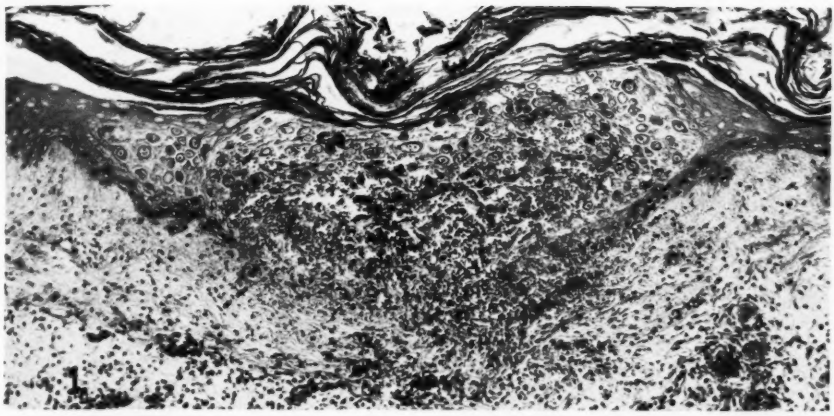
PLATE 86

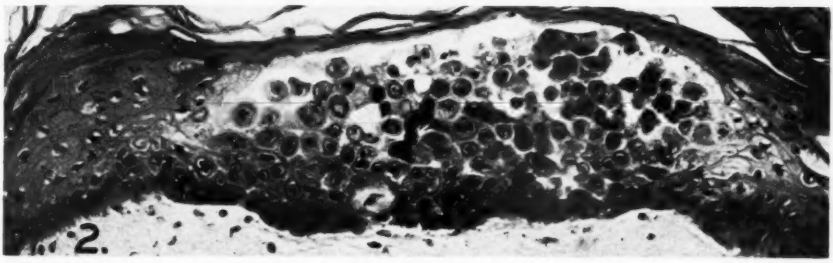
FIG. 1. Pustulating zoster lesion in human skin graft. (8 days.)

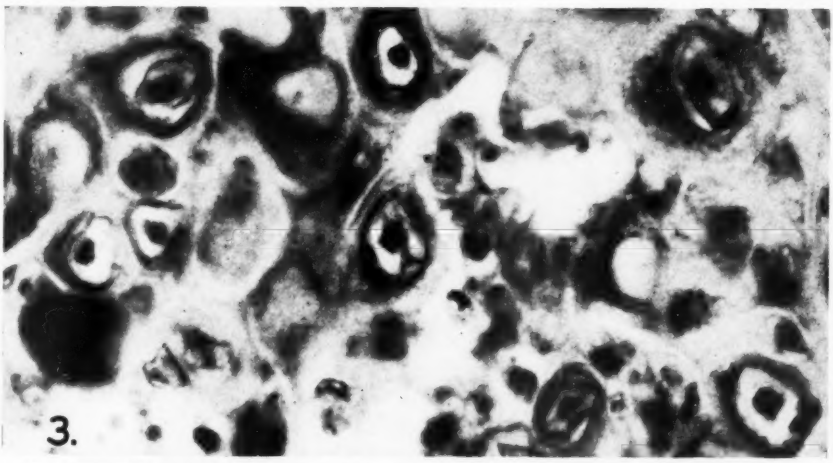
FIG. 2. Early (6 days) nonexudative zoster lesion in grafted human skin. Swollen, detached epithelial cells are shown, containing intranuclear inclusions. × 225.

FIG. 3. Intranuclear inclusions from the lesion shown in Figure 1. × 1200.

FIG. 4. Epithelial giant cell with intranuclear inclusions from Figure 1. × 1200.







Goodpasture and Anderson Herpes Zoster Virus in Grafted Human Skin

EXPERIMENTAL INFECTION OF THE CHICK EMBRYO WITH VIRULENT AND AVIRULENT PASTEURELLA PESTIS *

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The recent use of avirulent live strains of plague bacilli as vaccines for human immunization in several parts of the world has stimulated renewed interest in the biologic nature of this "avirulence." In earlier communications[1, 2] it was pointed out that virulence in plague apparently is independent of the possession of an envelope and that the most striking *characteristic of avirulent organisms is their inability to proliferate freely even in susceptible experimental animals,* although a complete permeation of the tissues of such animals can readily be observed. One of the principal difficulties of clear-cut differentiation between slightly virulent and completely avirulent organisms is the sliding scale of virulence that is observed when virulence tests of plague bacilli are performed in the common laboratory animals.

Buddingh and Womack[3] have studied the infection of the chick embryo with large doses of virulent *Pasteurella pestis.* Their findings indicate a high and uniform susceptibility of this host to plague infection. In order to investigate further the characteristics of the host-parasite relationship that may play a rôle in the determination of virulence or avirulence of plague bacilli, the experimental infection of chick embryos with known numbers of virulent and avirulent *P. pestis* was undertaken. This work involved the determination of relative susceptibility to, and reactions induced by, inoculation of various doses of plague bacilli into chick embryos. The observations on survival time, bacterial multiplication and persistence were supplemented by a histologic study of the changes resulting from infection with plague bacilli in the chorioallantoic membrane and the organs of the chick.

MATERIALS AND METHODS

The strains of *P. pestis* used in this study (virulent strain 106 and avirulent strain A1122) have been described previously.[2] Chick embryos of 11 to 14 days' incubation were inoculated with 0.1 ml. of broth culture dilutions in saline solution, containing a known number of live bacilli.[2] The technic followed was that of Goodpasture and Buddingh[4] for inoculation of the chorioallantois, while the methods of Polk, Buddingh and Goodpasture[5] and of Cox,[6] respectively, were used for

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injection of amniotic space or yolk sac. The inoculated eggs, together with suitable controls, were incubated at 37.5° C. and either observed for survival of the infection and hatching or killed at 24-hour intervals, the membrane and organs cultured[1] and pieces fixed in Zenker's fluid (with 4 per cent formaldehyde) for histologic sections. The sections were stained with hematoxylin and eosin as well as by Giemsa's method which permits the easy detection of plague bacilli in tissues.

Chicks that survived the infection with small numbers of avirulent *P. pestis* were killed at intervals after hatching and their organs cultured in order to determine the survival of avirulent bacilli. The culture methods used were the same as previously described.[1] In several experiments the practicability of serum protection tests with antiplague rabbit sera in chick embryos was tested. The influence of immune serum on the development and extent of lesions caused by virulent and avirulent plague bacilli was also studied. The antiplague immune serum which was produced by the injection of live avirulent organisms[2] had an anti-envelope agglutination titer of 1:320, and a protection titer of 25 provisional standard units.[7] One-tenth ml. of this serum diluted 1:2 was dropped onto the chorioallantois and was immediately followed by 0.1 ml. of the suspension of the infecting organism. The necessary controls with saline solution and normal serum were included in each test. The relative susceptibility of chick embryos, hatched chicks and white mice was determined by the subcutaneous or chorioallantoic injection of known numbers of organisms.

Experimental Results

When 12-day chick embryos were inoculated with graded doses containing from about 20 million to about 200 million plague bacilli, fairly striking differences were observed between the survival time and the hatching of chicks given virulent and those given avirulent *P. pestis*. A summary of the results obtained in several such experiments is presented in Table I. While there is no difference in the survival time of chick embryos infected with 200 million virulent or avirulent organisms, doses of 1 million organisms or less permit a significantly longer survival of chicks given avirulent bacilli. With doses of 5,000 or less avirulent *P. pestis* a few of the injected chicks regularly survived the infection and hatched at term. The percentage of hatching was never very large, but with such small numbers of avirulent bacilli (20 to 2,000) the average survival time of 12-day-old chicks was increased to almost 8 days after inoculation, since many of the animals died while hatching. Equally small numbers of virulent bacilli killed all chicks with an average survival time of 3.2 to 4.8 days.

The ability of avirulent *P. pestis* to persist and multiply in chick embryos without causing the latter's death was further investigated by killing chicks that hatched after infection with avirulent bacilli, and culturing their organisms. Some of the more important results obtained are presented in Table II. It was found that the number of bacilli in the infecting dose which permitted hatching of a significant proportion of chick embryos varied greatly with the route of administration and with the age of the embryo. Embryos less than 11 days old were quite highly susceptible and permitted the extensive multiplication of avirulent organisms leading to toxemia and death. Embryos of more than

TABLE I

Average Length of Life of Chick Embryos Infected with Equal Numbers of Virulent or Avirulent P. pestis

Approximate no. of organisms in infective dose	No. of 12-day chick embryos infected in each group	Average length of life* in days following inoculation with			
		Avirulent bacilli		Virulent bacilli	
		Days	% of embryos hatching	Days	% of embryos hatching
1 "loopful"	12	2.8	0	2.0	0
200 million	12	2.6	0	2.6	0
20 million	24	3.9	0	2.3	0
4 million	30	3.8	0	2.6	0
200 thousand	35	5.8	0	2.4	0
2 thousand	60	7.4	15	3.2	0
200	40	8.9	25	3.7	0
20	40	8.5	30	4.8	0

* Length of life of hatching chicks calculated as 11 days. Most of the dead embryos and some of the hatched chicks gave positive cultures when liver material was plated.

12 days' incubation usually died from large numbers of avirulent *P. pestis* but did not permit the survival of small numbers, so that no organisms could be recovered from the hatched chicks. Administration of the infecting dose on the chorioallantoic membrane or into the allantoic space was survived by some 12-day chicks only if less than 5,000 avirulent bacilli were given. Injection into the yolk sac, on the other hand, permitted the use of 500,000 avirulent *P. pestis* without unduly high mortality. The data presented in Table II were thus obtained under optimal conditions of infective dose (maximal) and age of chick embryos. It could be demonstrated that avirulent bacilli survive under suitable conditions for 3 days, on rare occasions for 4 days, after hatching of the chick, and can be recovered from the liver by simple culturing on blood agar plates and in gentian violet enrichment broth.[1] In spite of numerous attempts it was not possible to recover avirulent plague organisms after more than 4 days following hatching. This uniform disappearance of avirulent organisms around the fifth day after hatching suggested that a host-factor might be re-

TABLE II

Latent Infections in Chick Embryos Injected with Avirulent P. Pestis (Strain A1122) by Several Routes

Route of administration	Number of organisms administered	Number of chick embryos injected	Age of embryos in days when injected	Hatching		Plague organisms recovered from liver	
				Number	Per cent of total	Killed on day after hatching	Number positive over total number tested
Chorioallantoic membrane	3,000	38	14	10	26.3	1	6/10
Chorioallantoic membrane	3,000	83	12	17	20.5	3	3/8
						4	2/6
						5	0/3
Yolk sac	300,000	43	12	16	37.2	2	7/8
						3	2/4
						5	0/4
Allantoic space	3,000	35	14	8	22.8	3	1/6
						4	0/2
Allantoic space	300	30	13	8	26.6	2	2/5
						3	0/3

sponsible which was not present in a significant degree previous to that time. Such a host-factor might be evidenced by a high degree of resistance developing about the time of hatching. The susceptibility of 14-day chick embryos was therefore compared with that of newly hatched chicks, both with regard to infection with virulent organisms and to toxic extracts derived from *P. pestis*.[2] In several experiments it was found that 24 hours after hatching chicks were approximately one million times as resistant to infection with virulent or avirulent bacilli as 14-day-old chick embryos and that they withstood at least 10 mouse minimal lethal doses of toxic extract, *i.e.*, at least 100 chick embryo minimal lethal doses. The resistance of 24-hour-old chicks to infection was also spectacularly greater than that of mice, 30 million virulent organisms being necessary to kill all animals in a group upon subcutaneous injection. When avirulent strain A1122 was administered subcutaneously in doses of 100 million bacilli to groups of 1-day-old chicks, none of the animals died, and plague organisms could be recovered up

to the third day, only from the local lesions, but never from the liver. These findings were interpreted as probably indicative of the activation of a cellular mechanism a few days previous to, or at the time of, hatching.

There is reason to believe[7] that the action of antiplague serum in mouse protection tests depends, in part at least, upon the presence of a cellular mechanism that can be specifically activated. If such a mechanism were absent from 12-day chick embryos, then it could be predicted that immune serum of proved protective quality for mice should be without effect in chick embryos. This hypothesis was put

TABLE III

Serum Protection Tests in Chicks

	Strain	Number of organisms	0.1 cc. saline or antiserum dilution 1:2	Number hatching over total number in group	Average life in days after inoculation	Remarks
13-day chicks	106	3,000	Saline	0/12	5.7	With 106 in chicks the membranes were positive and livers positive
	106	3,000	Serum	1/12	7.9	
	A1122	300,000	Saline	3/12	7.3	With A1122 in some chicks the membrane was positive and liver negative, especially in those dying later
	A1122	3,000,000	Serum	4/12	7.6	
	A1122	3,000,000	Normal rabbit serum	2/10	7.9	Of 10 hatched chicks only 6 survived 48 hours; of these 6, 4 were positive at 48 hours after hatching
11-day chicks	A1122	3,000,000	Saline	0/17	2.7	
	A1122	3,000,000	Serum	0/16	5.1	All membranes and livers positive; most spleens positive
	106	30,000	Saline	0/17	2.0	
	106	30,000	Serum	0/17	3.1	

to test by inoculating 1 or 10 minimal lethal doses of virulent or avirulent organisms on the chorioallantoic membrane together with 2 provisional standard units of antiplague serum. Half this amount of serum will, on the average, protect mice against a lethal *P. pestis* infection as used in a standardized serum protection test.[7] The results of two such experiments are given in Table III. There was a slight increase in average survival time of serum-treated chick embryos over nontreated chick embryos, but it was statistically not significant in larger series. The occasionally observed longer survival time of serum-treated chick embryos infected with avirulent bacilli may possibly be due to the antitoxic properties of the serum. It is believed that these results tend

to confirm the concept of the absence of a significant cellular defense in the 12-day embryo and its appearance around the time of hatching.

Gross and Microscopic Observations of Pathologic Changes

The chorioallantoic membrane of 12-day-old chicks inoculated with 10,000 to 500,000 organisms of avirulent or virulent *P. pestis* showed the following changes: 24 to 48 hours following inoculation the membrane appeared edematous, and sometimes pin-point hemorrhages could be seen. These hemorrhages were much more pronounced after 60 hours if virulent organisms had been used for inoculation, and progressed steadily until death on the third to fourth day. With avirulent bacilli there were usually no hemorrhages to be seen from the third day on, but pock-like lesions appeared on the membrane which had a tendency to coalesce and form a marked central thickening. This elevation assumed first a whitish opacity, later a yellow coloring, and increased in firmness and height until either death or hatching. Its center frequently showed ulceration after the fifth day following inoculation of the chick embryo. Similar changes were seen if very small numbers of virulent bacilli were dropped on the chorioallantois, but with larger numbers (more than 5,000) the extensive hemorrhage dominated the picture and death usually occurred too soon for any proliferative changes to be noticed. In these cases the embryo also exhibited very marked subcutaneous hemorrhage, particularly on the head and neck, but the internal organs were only little involved, showing but slight enlargement, bright red color and no necroses. The visceral changes were altogether negligible in embryos inoculated with avirulent *P. pestis*. Even though it was possible to recover viable plague bacilli from spleen and liver of a number of embryos, these organs gave no microscopic evidence for reactive changes.

Comparative microscopic observations were carried out on several series of chick embryos (12 days old) inoculated on the chorioallantois with 30 or 300 plague bacilli. Control series injected with saline solution only provided the standard for comparison. Twelve-day embryos given about 30 virulent *P. pestis* organisms survived for 4 to 8 days. Twenty-four hours after inoculation the chorioallantoic membrane appeared slightly edematous, the capillary plexus seemed engorged and the ectodermal epithelium was proliferating slightly. There was no significant cellular reaction to be seen in the mesoderm. Liver and spleen appeared somewhat hyperemic. On the second day after inoculation the ectodermal proliferation had not progressed but the mesodermal edema was increased and there was some perivascular accumulation of large mononuclear cells. In a few places on the membrane extrava-

sation of erythrocytes could be noticed. The liver and spleen were unchanged. On the third day after injection bacilli could be observed microscopically for the first time. Some were seen within ectodermal cells of the epithelium which was not significantly hyperplastic, others between cells. Few only could be detected in the mesoderm within phagocytes or free. There were many mononuclear cells in the mesoderm, singly or in small groups, and particularly in perivascular "cuffing." On the fifth day after inoculation the membrane gave a picture of maximal edema with very intense cellular infiltration and large masses of bacilli throughout the basal regions of the mesoderm. In the upper layers of the mesoderm, in the vicinity of the capillary plexus, small heaps of bacilli were seen surrounded by densely packed mononuclear cells forming micro-abscesses. Few organisms, only, could be located definitely within phagocytes. There was very extensive multiplication of *P. pestis* in the liver, associated with cellular degeneration evidenced by loss of nuclear staining properties and slight vacuolization of some lobular margins. Relatively few mononuclear cells had infiltrated the liver sinusoids and the lymphocytic proliferation in the spleen was very moderate. In contrast to the finding of Buddingh and Womack,[3] however, the spleen was definitely the site of quite extensive focal bacterial localization and multiplication.

A similar series of eggs inoculated with about 300 virulent *P. pestis* organisms showed an identical sequence of events. All eggs in both of these series gave positive cultural results from the chorioallantoic membrane, and organisms could also be recovered from the livers of most of the embryos.

The inoculation of similarly small numbers of avirulent plague bacilli did not result in such histologic changes as just described for virulent bacilli. Most of the chick embryos survived until hatching term, following administration of 30 to 300 avirulent organisms on the chorioallantoic membrane, and about 30 per cent actually hatched while the rest were too weak to escape from the shell. Although it was possible to recover avirulent plague organisms from such chick embryos (from the chorioallantoic membrane and the liver) even 8 and 10 days after inoculation, histologic sections were almost indistinguishable from those prepared from an uninoculated control series. In a few instances there was a slight ectodermal proliferation and some monocytes could be seen in the mesoderm, but it was never possible to definitely identify bacilli in the sections and the reactive changes in either membrane or chick organs did not reach a significant level. Since the use of large avirulent inocula (about 1 million organisms) produces lesions in the chick embryo which resemble in all details those caused by small num-

bers of virulent bacilli, as previously described, the failure of small avirulent inocula to induce pathologic changes must be ascribed to their extremely slow rate of multiplication.

Histologic studies were also performed on chick embryos used for serum protection tests, mentioned previously. The reactions induced by 30,000 virulent organisms in 11-day-old chicks were fairly similar and varied but insignificantly with the treatment with saline solution or serum. After inoculation of either virulent or avirulent organisms and treatment with saline solution, the following changes could be observed regularly on the second or third day: The ectodermal epithelium was slightly hyperplastic and only a few bacilli could be seen in and between epithelial cells. The mesoderm appeared moderately edematous and its vessels engorged. There were a few small clumps of bacilli in the region of the capillary plexus but beneath it a solid mass of organisms extended through the deeper parts of the mesoderm. The inflammatory reaction to this bacterial invasion was negligible, with the appearance of only very few scattered mononuclear cells. A small number of bacilli were present in the liver without causing significant degenerative or inflammatory changes. In the case of chick embryos treated with hyperimmune antiplague serum before inoculation with avirulent organisms, the final picture was similar to that seen in nontreated chicks except for more extensive edema of the mesoderm, definite ulceration of the epithelium, and more pronounced mononuclear cell infiltration in membrane and liver. A number of serum-treated chicks dead on the second and third day after avirulent inoculation gave evidence for only limited bacterial invasion and multiplication. Death in these animals probably occurred due to the pronounced toxin sensitivity and a considerable amount of toxin liberated from the large inoculum after only slight multiplication. Serum-treated chicks given a virulent inoculum of 30,000 organisms did not differ from the untreated controls in any respect.

Discussion

It has not been possible up to the present time to distinguish virulent from avirulent *P. pestis* by means of serologic tests.[8] This may indicate either that there is actually no difference in antigenic make-up between these types of bacilli or that such a difference has as yet escaped detection. It was proposed recently[2] that the dissociation of virulent into avirulent plague bacilli may take place through a loss of antigenic constituent, chemical group, or property that either does not contribute to the exterior of the organism and thus would be difficult to determine, or may be highly unstable and easily destroyed. It

has been shown[1] that avirulent bacilli are to some extent invasive, pathogenic and toxic, so that these criteria are of no value for their differentiation from virulent organisms. No significant difference could be detected[9] between virulent and avirulent strains of *P. pestis* in either experiments evaluating coagulase and fibrinolysin activities or phagocytosis tests with artificially induced exudate. It appears, therefore, that the only striking characteristic that separates virulent from avirulent bacilli is the inability of the latter to proliferate freely, even in a highly susceptible or severely intoxicated host.[1] The results reported in this paper support such a view, for small numbers of avirulent organisms failed to give any evidence for significant multiplication in the highly susceptible chick embryo, while equally small inocula of virulent organisms multiplied extensively in a short time. This might be interpreted as indicating that avirulence may be a function of the loss of certain cellular enzyme systems which permit abundant multiplication in the animal host. It seems to be largely independent of the animal's defense mechanism, for studies[1,7] have shown that the effect of the immune animal or of exudative phagocytes mixed with immune serum is practically the same on virulent and avirulent plague bacilli. Furthermore, the 12-day-old chick embryo appears to be largely devoid of an efficient defense mechanism; nevertheless, the avirulent bacilli introduced in relatively small numbers do not proliferate freely. They do, however, persist until hatching time of the chick and sometimes even a few days longer, until the quickly developing defensive mechanism of the hatched animal disposes of them. This latent infection is somewhat similar to that reported by Oag[10] for *Borrelia duttoni*. Hatched chicks are extremely resistant to both intoxication and infection with *Pasteurella pestis* and are practically immune to the spirochete. Chick embryos are highly susceptible to both microorganisms and it appears that resistance develops rather suddenly upon hatching. The immaturity of the defensive mechanism in the chick is evidenced also by the fact that antisera of high protective value for mice are unable to protect embryos against plague infection. Furthermore, the histologic studies gave evidence for the low degree of defense, in the form of inflammatory response to plague infection, usually obtained in embryos.

In accordance with the findings in other experimental animals,[1] it was shown again that avirulent *P. pestis* inoculated in sufficiently large numbers can produce an invasion similar in all respects to that caused by virulent ones. The histologic observations made on eggs given small inocula of *P. pestis* emphasize the invasive features but fail to show the extensive ectodermal proliferation and destruction observed by

Buddingh and Womack[3] upon inoculation of "a loopful" of culture. This destruction as well as the "profuse hemorrhage" and "marked injury to the vessels" most likely is due to the toxic impact of the large bacterial mass and does not occur if smaller, primarily nontoxic doses of organisms are used. The blood stream invasion of the embryo regularly led to the development of foci not only in the liver[3] but also in the spleen, both organs filtering plague bacilli out of the blood coming from the chorioallantoic membrane. The reaction of the embryo is approximately the same to equal large doses of virulent and avirulent *P. pestis* so that a histologic differentiation is impossible. It appears probable that the final criterion of virulence will have to rest on a biologic and chemical identification of the bacterial enzyme systems which are responsible for multiplication of plague bacilli in the animal host.

Summary and Conclusions

1. Chick embryos of 12 to 14 days' incubation are very highly susceptible to infection with *P. pestis*. Twenty virulent, or 200,000 avirulent plague organisms bring about death of the embryos in 4 to 8 days. In spite of this high susceptibility, avirulent plague bacilli inoculated in small numbers fail to proliferate freely. The lack of abundant multiplication even in a highly susceptible host is considered an inherent characteristic of completely avirulent *P. pestis* organisms.

2. Avirulent plague bacilli administered onto the chorioallantoic membrane of 12-day-old chick embryos in sublethal doses persist in the embryo's organs until hatching time and sometimes for 3 to 4 days after hatching of the chick.

3. Newly hatched chicks are very highly resistant to plague infection and large doses of virulent organisms are disposed of rapidly, probably through the agency of a cellular mechanism that is activated at hatching time. The absence of such a mechanism in 12 to 14-day-old chick embryos may be responsible for their high susceptibility.

4. Hyperimmune plague antiserum of known protective quality for mice is unable to protect chick embryos against an infective dose of *P. pestis*. This is taken to indicate that a cellular defense mechanism must be present for antiserum to exert its protective action. In the absence of such a mechanism only the antitoxic, but not the antiinfectious, activity of antiserum becomes evident.

5. Histologic observations indicate that virulent *P. pestis* are distributed from the membrane by way of the blood stream to all organs of the embryo and produce in them degenerative lesions. The proliferative response to either virulent or avirulent plague bacilli in the embryo's organs appears slight. Ulceration of the ectoderm and vas-

cular damage occurs only when very large inocula are used and is an expression of toxic action.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 87

FIG. 1. Chorioallantoic membrane from a 15-day-old chick embryo, inoculated on the 12th day with about 30 virulent *Pasteurella pestis* organisms. There is very slight ectodermal proliferation but marked mesodermal edema. Giemsa's stain. × 70.

FIG. 2. Detail from Figure 1 showing a small cluster of plague bacilli in the mesoderm of the membrane, with some mononuclear cells. Giemsa's stain. × 635.

FIG. 3. Chorioallantoic membrane from a 14-day-old chick embryo, inoculated on the 11th day with 30,000 virulent plague bacilli. There is no significant proliferation of the ectoderm and but negligible mesodermal edema, but there has been massive multiplication of organisms in the basal parts of the mesoderm. All vessels are engorged. Giemsa's stain. × 20.

FIG. 4. Membrane from a chick embryo treated similarly to that shown in Figure 3, but given in addition hyperimmune plague antiserum simultaneously with the infective dose. More marked edema than shown in Figure 3, intense hyperemia, and massive bacterial proliferation in the basal regions of the mesoderm are present. Giemsa's stain. × 20.

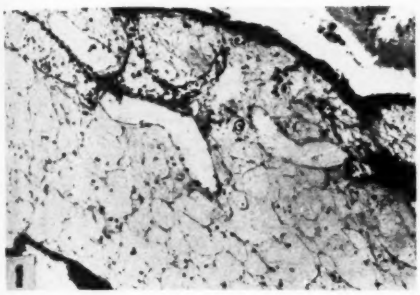
FIG. 5. Liver from the same chick embryo as that shown in Figure 3. Focal bacterial proliferation and slight degenerative changes are seen in the lobules adjoining such foci. Giemsa's stain. × 70.

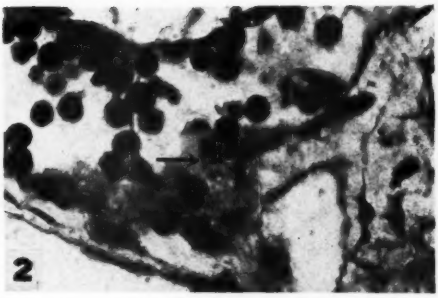
FIG. 6. Detail from Figure 5, showing a focus of bacterial multiplication in the liver parenchyma and simultaneous intravascular proliferation. Giemsa's stain. × 320.

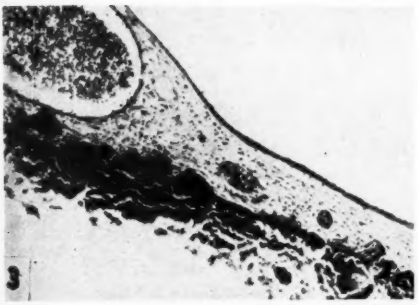
FIG. 7. Spleen from the same chick embryo as that shown in Figure 3. Several foci of bacterial multiplication are shown in the pulp. Giemsa's stain. × 70.

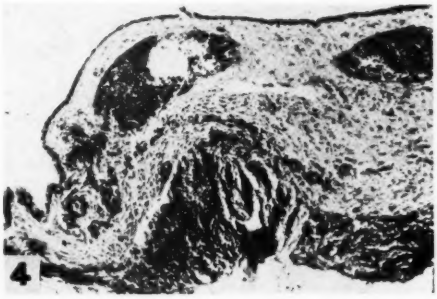
FIG. 8. Detail from Figure 7, showing one small mass of multiplying organisms in the splenic pulp and a number of single organisms both intercellular and intracellular. Giemsa's stain. × 635.

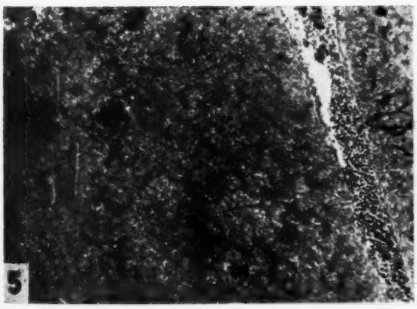
FIG. 9. Detail from Figure 5. Bacteria are carried by the blood stream, free and in white cells. One clump of multiplying organisms adheres to the endothelium of a hepatic vein. Giemsa's stain. × 635.

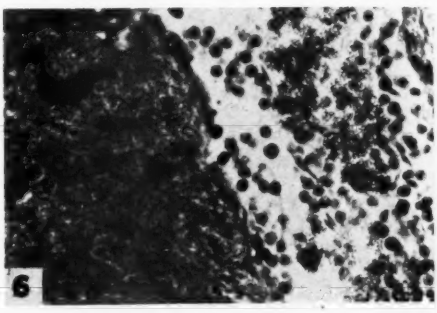


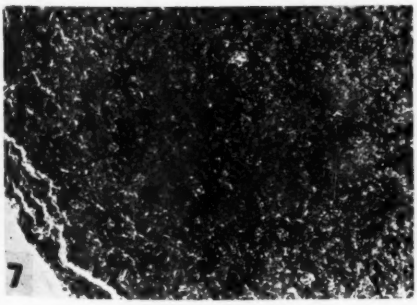


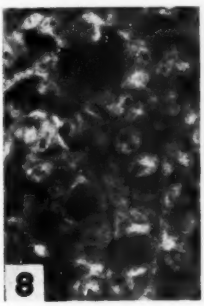


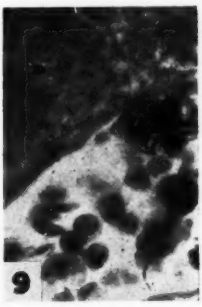












Jawetz and Meyer *Pasteurella pestis* in the Chick Embryo

THE PATHOLOGY OF FATAL EPIDEMIC HEPATITIS *

BALDUIN LUCKÉ, Lt. Col., M.C.

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INTRODUCTION

During the spring and summer of 1942 an outbreak of jaundice of considerable magnitude occurred in the Army of the United States. General information concerning this outbreak has been given by the Surgeon General in a circular letter which was published in medical journals.[1] As stated in this circular, groups of investigators were assigned the study of various aspects of the disease; investigation of its pathology was conducted in the laboratories of the Army Medical Museum, where material from all available sources was assembled. This paper is based upon a study of 125 cases; the patients included

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the military personnel and, in addition, a number of civilians who died during the outbreak. The contents and arrangement of the paper are given above. The study does not include a consideration of the etiology, nor of the clinical manifestations of nonfatal cases; these aspects are dealt with by others,[2-4] in reports in which the relation of the administration of yellow fever vaccine, containing human serum, to the outbreak of hepatitis and jaundice are considered in detail.

Historical

Epidemics of Hepatitis. The disease which is variously known as epidemic hepatitis, infective or infectious hepatitis, or epidemic catarrhal jaundice is not new. Outbreaks with similar clinical and pathologic manifestations have occurred for at least the past 100 years.[5-8]

The disease has always been prevalent in armies, and particularly so in times of war. Hence English writers have referred to it as camp jaundice, or epidemic catarrhal jaundice of campaigns,[9] French writers as "jaunisse de champs," [10] German writers as a "Kriegs" icterus catarrhalis, or "Soldatenkrankheit" icterus.[11, 12]

One of the largest of recorded epidemics occurred in this country during the Civil War.[13] During the first year there were 10,922 cases with 40 deaths among the Federal troops (the mean strength of the U. S. Army being 279,371 men). During the second year 32,154 cases occurred with 119 deaths (mean strength of Army, 614,325 men). During the third year the epidemic declined; there were 9057 cases and 67 deaths (mean strength, 619,703). In the fourth year the number of cases had fallen to 294 with 5 deaths (mean strength of Army, 89,143). Woodward [14] in his book on "Outlines of the Chief Camp Diseases of the U. S. Armies," published in 1863, gives an excellent account of the clinical course of the disease.

"When this form of jaundice attacks a regiment or an Army, it usually appears in a number of cases simultaneously or in close succession like other epidemic disorders, lasts in each case from one to six weeks, or even longer, and then slowly disappears. The appearance of the icteroid hue is, as a rule, preceded by more or less derangement of the general health; sometimes, however, only by a few days of headache, constipation, and malaise, and occasionally the discoloration of conjunctiva and skin is the first noticeable symptom. The color of the skin may vary from a scarcely noticeable tinge to a deep tawny-orange color; this condition is accompanied by depressed spirits, intellectual torpor, loss of appetite, general debility, and uneasy sensations over the region of the liver and the stomach. Hepatic tenderness is a very variable symptom; enlargement of the liver, as indicated by an increased area of dullness on percussion, is more common. Sometimes there is nausea and vomiting. The stools are usually clay colored, and the bowels constipated, though at times there is diarrhoea. The urine is discolored from the presence of biliary matters. Very often the patient is so debilitated as to be quite unfit for duty, though not usually confined to his bed; at other times, however, he continues to perform service throughout the affection.

"After lasting a variable period, the symptoms slowly subside and the patient is gradually restored to health, the mental torpor and debility persisting often some time after the icteroid hue has disappeared. The first symptom of amendment is generally a change in the color of the stools, which gradually resume their normal appearance. Cases occasionally occur of a graver character than indicated above, the symptoms of biliary toxemia being aggravated to stupor or even coma, and such cases at times prove fatal."

This description of the epidemic of 1862 gives a good picture of the epidemic of 1942. Indeed, with minor variations, the clinical manifestations in the many epidemics have been remarkably similar.

During the Franco-Prussian War there were 2344 cases in the Prussian Army, 1311 cases in the Bavarian and 407 cases in the Saxon Army.[15, 16] During the South African War 5,648 cases were recorded.[10] In World War I, epidemics occurred in the armies of several nations. Among the British, so-called catarrhal jaundice first broke out in July, 1915, among troops stationed in Egypt; thereafter the epidemic spread rapidly to Gallipoli, where 2195 cases occurred from September to November, and then to Mesopotamia, where 1538 British and 2634 Indian troops were affected.[9, 17] The disease was as frequent among the French as among the British, but no cases occurred among the Turks. Among Rumanian troops there was a serious epidemic in the fall of 1917; one writer speaks of it as taking place in an almost explosive manner. Several thousand men were affected.[18] (See this paper for references to outbreaks among German troops.) No noteworthy epidemic occurred in the American Army during the war, but a relatively small outbreak developed in the Army of Occupation.[19]

Since the war, epidemic hepatitis has become widely prevalent among the populations of several countries. Blumer [20] recorded many outbreaks in the United States, of which more than 200 were observed during 1921 and 1922 in the State of New York alone. Cullinan [21] gave a list of reported outbreaks between 1926 and 1939 in England. Ruge [22] recorded an extraordinary rise in incidence of hepatitis between 1919 and 1929 in the German Navy. But it is in the Scandinavian countries that the disease became especially widespread and virulent. The extensive literature dealing with these Scandinavian epidemics is collected in the papers by Ehrström, [23] Wallgren,[24] Wickström [25] and Selander.[26]

In the present War, as in previous wars, the disease has at times been prevalent in the combatant forces: in the Army of the United States,[1] in British Forces [27, 28] and in the German Army.[12, 29,30] In discussing the occurrence of hepatitis in the German Army, Dietrich,[12] in 1942, stated that it had become one of the most important epidemic diseases.

Terminology. Until recent years, the disease under consideration was universally spoken of as catarrhal jaundice. This term reflected the views of Virchow[31] concerning its pathogenesis and nature. He believed that catarrhal jaundice is caused by inflammatory swelling of the orifice of the common bile duct, which, he thought, became obstructed by a plug of mucus and desquamated epithelium. Although he gave detailed directions for opening the duct so as to observe the occluding plug, the reader of his paper is left in doubt how often Virchow observed such an obstruction. In the course of time the correctness of Virchow's view was questioned by many clinicians, and the hypothesis was proposed that the site of the essential lesion is not in the extrahepatic bile ducts but in the liver.[32] So-called catarrhal jaundice gradually came to be regarded as an inflammatory process in the liver itself, *i.e.*, a form of hepatitis.

The term "epidemic hepatitis" was first applied by Lindstedt[33] in 1918 to the epidemic form of "catarrhal jaundice"; he proposed this term in contradistinction to "infectious hepatitis" or Weil's disease. Lindstedt's nomenclature has been followed particularly in the Scandinavian countries and on the European continent. In the United States and in Great Britain various terms are in use: infective jaundice, catarrhal jaundice, so-called catarrhal jaundice, infectious hepatitis, infective hepatitis, simple hepatitis, and epidemic hepatitis. None of these terms is wholly satisfactory. Probably not until the discovery of its etiologic agent will the disease receive a definitive name. In this paper, following the rules of priority in nomenclature, the disease will be referred to as epidemic hepatitis.

The Relation Between the Sporadic (Endemic) and the Epidemic Form of Hepatitis. It is the consensus at present that epidemic hepatitis is the epidemic form of the disease which in sporadic cases has hitherto been called catarrhal jaundice.[7, 24-26] The sporadic and epidemic forms are related in somewhat the same way as are sporadic and epidemic poliomyelitis or influenza.[5, 7] Many epidemics of hepatitis have been traced to sporadic cases, and the two forms cannot be distinguished on either clinical or epidemiologic grounds.[7]

Not every student of this disease, however, shares the view that the two forms are identical. Thus Selander[26] in his recent monograph stated that although the two forms have a very similar clinical picture, he yet considers sporadic catarrhal jaundice and epidemic hepatitis as two different diseases. He based his opinion on the more gradual development of the sporadic disease, which tends to affect adults, whereas the more rapidly developing epidemic form tends to affect children. Dietrich,[12] on the other hand, came to the conclusion that any differences in development or course merely represent reactions at two dif-

ferent age periods. All investigators agree that the question cannot be settled conclusively until the etiology of epidemic hepatitis is established.

Mortality. Like the sporadic form, epidemic hepatitis usually runs a mild course. The mortality is low; the rates in various epidemics, including the outbreak of 1942, have ranged from 0.13 to 0.44 per cent. During the Civil War there were 52,429 reported cases of jaundice and 231 deaths, with a mortality rate of 0.44 per cent.[13] During the previous World War, 1538 British and 2634 Indian troops stationed in Mesopotamia were affected, with a mortality of 0.4 per cent.[17] The incidence in the German Navy between 1919 and 1929 was approximately 2500, with a mortality of 0.13 per cent.[22] During a large epidemic in Finland, between 1933 and 1936, the mortality was 0.34 per cent.[24] Selander [26] estimated the mortality in recent Swedish epidemics as from 0.2 to 0.4 per cent.

In conformity with the policies of the War Department, specific statements concerning numbers of cases must for the present be omitted. It is possible, however, to state that in the 1942 outbreak the mortality was 0.24 per cent, *i.e.*, even lower than in most other recorded epidemics.

Pathology. Until the previous World War there was no precise information as to the lesions of catarrhal jaundice. During the war Eppinger [34] performed autopsies on three soldiers who died of trauma. The liver showed lesions which he regarded as a miniature form of acute yellow atrophy. For the first time it was shown by morphologic examination that catarrhal jaundice is in fact a disease of the liver. There was no obstruction of the extrahepatic biliary passages.

There have been but few other post-mortem examinations; a case was reported by Klemperer, Killian and Heyd,[35] one by Gaskell,[36] another by Schrumpf,[37] one by Barber and Osborn.[38] All confirmed Eppinger's findings that catarrhal jaundice is a disease which causes destructive changes in the liver.

To this meager information much has been added by the studies of Roholm and Iversen [39] and by those of Dible, McMichael and Sherlock.[40] These investigators examined material obtained for biopsy by aspiration of the liver during various stages of the disease. It became certain that catarrhal jaundice is a form of hepatitis.

Relation of Epidemic Hepatitis (Catarrhal Jaundice) to Idiopathic Yellow Atrophy. The studies previously mentioned deal with the lesion occurring in the usually benign, nonfatal case. During large epidemics, when perhaps the disease becomes more virulent, a much greater number of patients have been examined post-mortem. Invariably, the liver has shown the changes of idiopathic yellow atrophy. As early as 1912

Cockayne [5] reviewed the evidence for linking these two diseases which generally had been regarded as unrelated. He came to the conclusion that catarrhal jaundice and yellow atrophy are usually due to the same cause, a specific organism of unknown nature. Since the last war, many observers have commented on the increasing frequency of catarrhal jaundice, in both its sporadic and epidemic forms, and on the greater frequency with which acute yellow atrophy (not related to poisons or bacterial infection) has appeared at the autopsy table. Most of the cases of idiopathic yellow atrophy have occurred during or shortly following an epidemic of catarrhal jaundice. In the Scandinavian countries and in Great Britain both diseases seem to have been more prevalent than elsewhere; and in these countries many investigators have taken the view that yellow atrophy and benign catarrhal jaundice are but two extremes of one and the same disease.[5, 7]

This view is not shared by all investigators. During the serious outbreak of hepatitis in Sweden during 1927, Bergstrand [41] studied 95 fatal cases. Yellow atrophy of the liver was found in all. He regarded yellow atrophy merely as a complication of epidemic hepatitis. Bergstrand gave no adequate grounds for making this distinction, and his views were not generally accepted.

During the present war, several papers have dealt with the pathology of fatal epidemic hepatitis: Fox, Manso, Penna and Madureira Pará [42] reported 17 cases from Brazil, Cameron [27] reported 4 fatal cases from Palestine, and Siegmund [30] 3 from Germany. Yellow atrophy of the liver was the common finding in all.

The present study supports the view that idiopathic yellow atrophy represents the extreme lesion of epidemic hepatitis. It remains to be emphasized that this form of yellow atrophy differs anatomically from the yellow atrophies caused by arsenic, phosphorus and a large number of other chemical agents. It also differs from the yellow atrophy of eclampsia, and that of a number of bacterial infections, particularly those involving the peritoneum.

Material and Methods

The material available comprised clinical records, autopsy protocols and fixed tissues from 125 cases. In approximately two-thirds of the cases the complete clinical data were examined; in the remainder, more or less complete abstracts of the records. All cases had been studied in hospitals, and all had been diagnosed clinically as epidemic hepatitis (or as epidemic catarrhal jaundice). Most of the post-mortem examinations were performed by Army Medical Officers; their cooperation has greatly facilitated this study.

Paraffin sections were prepared from all tissues, and those of the liver were stained with hematoxylin and eosin, by the Masson-Mallory method for connective tissue and by Wilder's method for reticulum. In many instances various fat stains were used, and the Giemsa and the MacCallum stain for bacteria. Sections of organs other than liver were usually stained with hematoxylin and eosin, and frequently by the other methods. Frozen sections of the brain in selected cases were stained by Cajal's gold sublimate method and by Hortega's method for glia; Nissl stains, hematoxylin and eosin and myelin stains were used for paraffin sections.

Clinical Course of Fatal Epidemic Hepatitis

Epidemic hepatitis usually is a mild disease; its clinical course is well known and has been described in numerous papers. There is much less information concerning the course of hepatitis that terminates fatally. Therefore, in the present section an analysis is given of the clinical course of 125 fatal cases. In this analysis special emphasis is laid on the stages of the disease, on the times at which jaundice, ascites and nervous manifestations appeared, and on the more important laboratory findings. The data will be presented in the form of tables and graphs, so that only a brief summary need be given in the text. Abstracts of representative clinical records will be used to illustrate these data.

Age, Sex and Race of 125 Patients with Epidemic Hepatitis

The pertinent data are given in Table I. In the present series all except 4 patients were males; 94 per cent were whites, 6 per cent were colored. This distribution probably reflects the population in the Army, although no comparative figures are at present available. Seventy-

Table I

Age, Sex and Race of 125 Patients with Epidemic Hepatitis

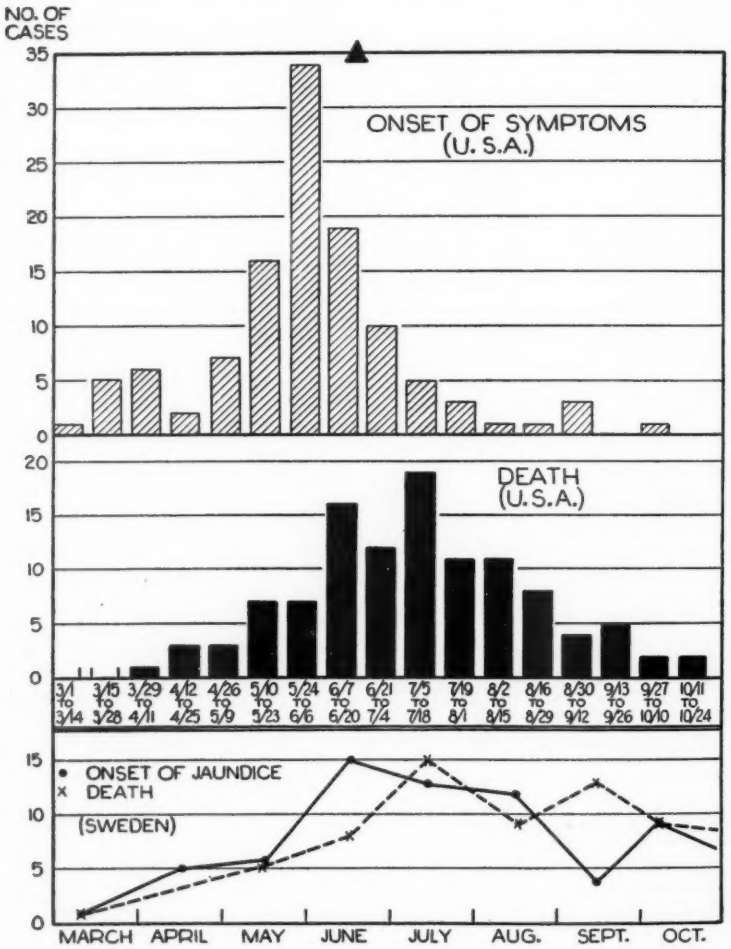
Age	Number of patients		
	White	Colored	Total
(*years*)			
20–24	*46 (37%)	4 (3%)	50 (40%)
25–29	*45 (36%)	2 (2%)	47 (38%)
30–34	9 (7%)	0	9 (7%)
35–39	11 (9%)	1 (1%)	12 (10%)
40–44	5 (4%)	0	5 (4%)
45–49	0	0	0
50–54	2 (2%)	0	2 (2%)
Total	118 (94%)	7 (6%)	125

All except 4 patients were males.
* Includes 2 females.

eight per cent were below 30 years of age. It is of interest to point out that fatal epidemic hepatitis was not entirely confined to the younger age groups; 22 per cent of the patients were above the age of 30.

Relation of Season to Onset of Symptoms and Death

The outbreak of hepatitis began in March, reached its peak toward the end of June and thereafter gradually declined, so that by the end



Text-Figure 1

of August few new cases were developing. It seems of interest to compare the onset of symptoms and the time of death of the fatal cases, first, with those of the entire outbreak and, secondly, with those in the

great Swedish epidemic of 1927. These relations are shown graphically in Text-Figure 1. Here the onset of symptoms and the time of death have been graphed in bi-weekly periods. It will be seen that the peak of onset of symptoms in fatal cases (shown in upper part of the figure) corresponds closely with the peak for all the cases of the epidemic (this peak is indicated by a solid triangle on the top line of the graph).

The modes in these two graphs are approximately 4 to 6 weeks apart.

TABLE II

Duration of Disease in 118 Cases of Epidemic Hepatitis

Duration	No. of cases	Per cent of cases
(*days*)		
Less than 10	0	0
10–19	14	11
20–29	20	16
30–39	31	26.
40–49	20	17
50–59	8	7
60–69	8	7
70–79	9	8
80–89	0	0
90–99	5	5
Over 100	3	3

That this length of time represents the most frequent duration of the disease is confirmed by the data given in Table II.

A similar relation between onset and time of death is shown for the Swedish epidemic at the bottom of Text-Figure 1.

Course of Fatal Hepatitis

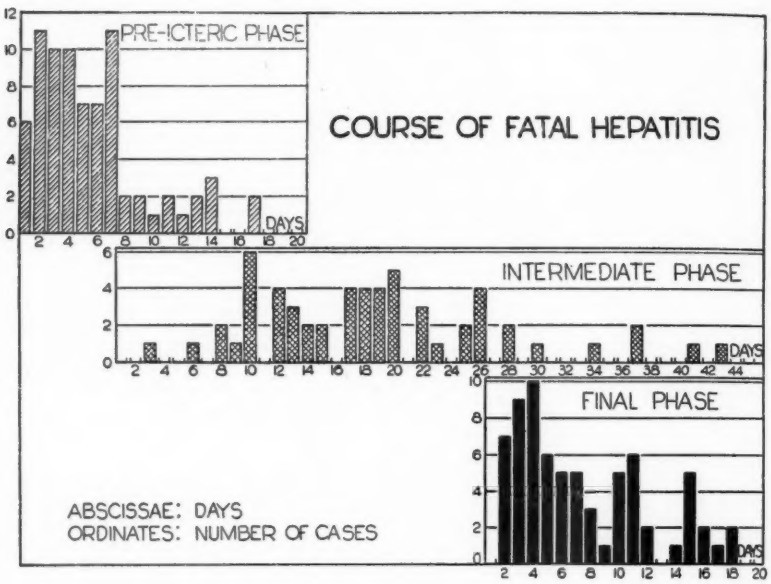
Three distinct phases in the clinical course of fatal hepatitis may be recognized: a pre-icteric, an intermediate and a final phase. The intermediate phase begins with the onset of jaundice and, in these fatal cases, usually ends abruptly with the appearance of new grave symptoms that presage the fatal termination. The time relation of the three phases is shown in a graph (Text-Fig. 2), where the ordinates give the number of cases, and the abscissae the duration. The pre-icteric phase in the great majority of cases lasted 7 days or less, and shows little scattering (see also Table III). In the intermediate phase, however, there is considerable scattering, though in the majority of cases the

TABLE III

Interval Between Onset of Symptoms and Appearance of Jaundice in 77 Cases of Epidemic Hepatitis

Interval	No. of cases
(*days*)	
1–4	37
5–9	29
10–14	9
15–19	2

duration was 26 days or less. The final phase, in the majority of cases, ran a course of 10 days or less.



Text-Figure 2

Initial Symptoms. The initial symptoms, *i.e.*, those of the pre-icteric stage, are presented in Table IV. The most common initial manifestations of the disease are anorexia, nausea, dark urine, abdominal

TABLE IV

Initial Symptoms in 120 Cases of Epidemic Hepatitis

Symptom	No. of cases	Per cent of cases
Anorexia	85	71
Nausea	62	52
Dark urine	39	33
Abdominal distress	37	31
Vomiting	29	24
Malaise	25	21
Weakness	24	20
Constipation	14	12
Headache	14	12
Fatigue	10	8
Backache	5	4
Coryza	3	3
Pruritus	3	3
Diarrhea	3	3
Chill	2	2
Urticaria	2	2
Epistaxis	1	1
Pain in joints	1	1
No pre-icteric symptoms	1	1

distress, vomiting, malaise and weakness. It should be emphasized that whereas approximately half the patients gave a history of nausea, only a quarter of the patients vomited.

Intermediate Phase. In the majority of cases the clinical picture in the intermediate phase gave no indication that the disease was not going to run the usual benign course. Many patients were ambulatory. In many instances the records contain statements such as "not acutely ill," "general condition good," "appears to be doing well," "uneventful course." In a considerable number the initial symptoms abated and the patients appeared to be steadily improving. Only in the exceptional case was the course grave from the beginning.

TABLE V

Duration of Final Phase in 73 Cases of Epidemic Hepatitis

Duration	No. of cases
(*days*)	
Up to 4	26
5–9	21
10–14	13
15–19	10
20 and over	3

Final Phase. A sudden dramatic change for the worse in 73 cases ushered in the final phase. The characteristic changes were nervous symptoms, ascites and persistent vomiting. The duration of this phase is shown in Table V. In over 60 per cent of the cases, death occurred within 10 days from the appearance of grave symptoms. Only 3 patients survived longer than 20 days (Table V).

Nervous Manifestations. In the great majority of cases the patients had signs of cerebral involvement. Particularly noteworthy were lethargy or coma, alternating with restlessness, excitement and delirium. Other manifestations were scanning speech, muscular weakness, and exaggeration of deep and superficial reflexes. Nervous manifestations usually ushered in the final phase; they marked the turning point. In a few cases, however, these manifestations appeared earlier or else were agonal (Table VI). The clinical course is illustrated by representative cases.

TABLE VI

Interval Between Onset of Nervous Manifestations and Death in 88 Cases of Epidemic Hepatitis

Interval	No. of cases	Per cent of cases
(*days*)		
Up to 4	53	60
5–9	19	22
10–14	8	9
15–19	6	7
20 and over	2	2

Illustrative Cases

Case 8

Clinical Course. The patient was a white male, 28 years old, who was well until the end of March, 1942. He then had anorexia, nausea and diarrhea; later, abdominal distress. On April 2, there was slight jaundice, but he was not ill and continued on duty until April 25, when he was admitted to hospital. Temperature, pulse and respiration were normal; liver enlarged, tender; urine, persistent traces of albumin and bile; stools, clay-colored. May 5: poor appetite; complained of being light-headed and dizzy; liver, enlarged and tender. May 8: slightly stuporous, somewhat confused. May 11: rapidly deepening stupor; sharp piercing cries; blood pressure, 150/85; disoriented and irrational; rapid change for the worse; alternating periods of coma and delirium. May 15: very toxic and delirious; did not respond to questions. May 16: comatose; rapid decline; vomited brownish material; died on this date.

Case 13

Clinical Course. The patient was a white male, 26 years old. On April 4, 1942, there was anorexia and later vomiting. April 9: jaundiced. Admitted on April 29, deeply jaundiced, tired, responsive; no fever; pulse, 76; abdomen flat; liver not palpable but slightly tender. May 5: temperature rose to 101° F.; blood pressure, 140/80; markedly dehydrated. May 6: somewhat stuporous; intermittent spasm of left sternocleidomastoid muscle; jerked head from side to side; tendon reflexes hyperactive but approximately equal on the two sides; sustained ankle clonus; Babinski reflex negative. May 17: abdomen distended; paracentesis, 600 cc. of fluid withdrawn. May 19: paracentesis, 1500 cc. of fluid withdrawn. May 21: paracentesis, 1500 cc. of fluid withdrawn. May 22: stupor progressed into coma. May 23: died.

Laboratory Findings.

Urinalyses

Date	Color	Specific gravity	Albumin	Acetone	Sugar	Bile
April 30	Dark	1.011	−		+	+
April 30	Coffee	1.015	+		−	+
May 1			−		−	+
May 2			+			++
May 5	Coffee	1.010	Faint trace	−	−	++
May 6						++

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Neutrophils (*per cent*)	Lymphocytes (*per cent*)	Monocytes (*per cent*)	Eosinophils (*per cent*)
April 30	4.85	9,300	59	27	2	1
May 1	5.48	7,200	60	23	12	2
May 4	5.62	7,450	48	42	9	
May 5	5.31	5,850	56	30	10	2

Examination of Feces

Date	
May 1	Gray, no bile present
May 6	Clay-colored

Chemical Examination of Blood

Date	Icterus index	Van den Bergh reaction	Serum protein Total (*gm. %*)	Albumin (*gm. %*)	Globulin (*gm. %*)	A/G ratio
May 2	164	+ (direct)				
May 3	132	+ (direct)				
May 5	120	+ (direct)				
May 25	120		5.4	1.5	3.9	0.45

Case 27

Clinical Course. The patient was a white male, 33 years of age. On May 13, he complained of headache, nausea and malaise. May 20: "dark" urine. May 23: light-colored stools; moderate degree of jaundice. Upon admission on May 23, did not appear acutely ill; liver moderately tender. May 26: no complaints. May 29: appetite decreased, jaundice more marked. Condition remained the same until June 3, when liver became moderately enlarged, tender. June 5: considerable epigastric distress. June 9: became delirious and irrational, slight convulsive movements; blood pressure, 116/64; temperature, pulse and respiration normal. June 11: after return to normal mental state, again extremely restless and semi-delirious; spasmodic movements at intervals, such as jerking of head and contracting of abdominal muscles, not convulsive; at times answered questions rationally, at other times was irrational; intensely jaundiced; no vomiting; later in day became very drowsy, fell asleep while examined. June 12: delirium persisted, noisy, very restless; cried out as if in pain; later comatose. June 13: condition essentially unchanged. June 14: much improved; responded to questions and was rational for short periods. June 15: again delirious; positive Babinski reflex; ankle clonus. June 16: vomited coffee-ground material; later, deep coma. June 17: bronchopneumonia evident; condition very poor. May 23 to June 11: temperature, 97.6° to 98.4° F.; pulse, 68 to 80; respiration, 18 to 20. June 11 to June 17: slightly febrile, temperature was 99.2° to 100.0° F. June 17: died.

Laboratory Findings.

Chemical Examination of Blood

Date	Icterus index	Nonprotein nitrogen (*mg. %*)	Sugar (*mg. %*)	Serum protein Total (*gm. %*)	Albumin (*gm. %*)	Globulin (*gm. %*)
May 26	42					
June 11	200	40	62			
June 12		44	70	6.2	3.0	3.2
June 15		45	112			
June 16	160					

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes (*per cent*)
June 12	4.8	10,000	81	16
June 16	4.5	10,000		

Urinalyses

Date	Albumin	Bile
June 12	+	+
June 15	+	+
June 16	+	+

Case 76

Clinical Course. The patient was a white female civilian, 27 years old. She had not received yellow fever vaccine. In April, her husband, an officer, had a mild attack of hepatitis which lasted about 1 month. The patient herself was well until August 1, 1942, when general malaise developed and her appetite began to fail. August 25: jaundice developed. Admitted on September 5, moderately jaundiced; blood pressure, 118/78; liver tender and moderately enlarged. During her early stay in the hospital was occasionally jaundiced but felt fairly well; appetite was good. September 9: jaundice had deepened; definite nausea. Uneventful, afebrile course with small improvement until October 11, when swelling of face developed due to infection of right lower molar tooth; slight irregular fever during this period; jaundice was decreasing. October 15: diplopia developed and continued. October

28: speech became like that of an intoxicated person; normal clear-cut pronunciation altered so that her words were difficult to understand. October 30: very emotional, alternating periods of euphoria and crying; disoriented with regard to time, but oriented with regard to place and person; speech thick and slurred; deep and superficial reflexes normal, no abnormal reflexes could be demonstrated; general decrease in motor power and muscle tone which was attributed to poor physical condition rather than to involvement of central nervous system; clinical impression of acute toxic encephalitis. October 31: violently delirious, screamed and tried to get out of bed; unable to take food by mouth. November 2: twitching movements of hands and feet. There had been a gradual rise in temperature to 106° F. from October 30 to November 4. November 4: died.

Laboratory Findings.

Blood Counts

Date	Red blood cells (*millions*)	White blood cells	Polymorphonuclear leukocytes (*per cent*)	Lymphocytes (*per cent*)
Sept. 5	3.4	4,300	65	30
Oct. 20	2.7	4,400	69	31
Oct. 22	2.7	4,700	70	27
Nov. 2	2.8	11,600	89	7

Chemical Examination of Blood

Date	Icterus index	Urea nitrogen (*mg. %*)	Nonprotein nitrogen (*mg. %*)	Serum protein Total (*gm. %*)	Albumin (*gm. %*)	Globulin (*gm. %*)
Sept. 7	100					
Sept. 9	109					
Sept. 14	123					
Sept. 25	154					
Oct. 5	147					
Oct. 12	156					
Oct. 29	76					
Nov. 3	116	34	64	7.2	3.9	3.3

Urinalyses

During early stay in hospital, no albumin; later, trace to moderate amount.

Case 121

Clinical Course. The patient was a white female civilian, 23 years of age, who had not received yellow fever vaccine. About December 1, 1942, there was dyspepsia, constipation, lassitude, progressive weakness; suddenly became jaundiced. December 18: admitted. December 19: temperature, 98° F.; pulse, 80; respiration, 18; blood pressure, 110/70; albuminuria; jaundice cleared considerably. By January 22, icterus index had fallen to 148. General condition improved; patient returned to her home. Upon readmittance on January 28, abdomen was distended; moderate irregular fever; complained of general aching. February 6: paracentesis, 1600 cc. of bile-stained fluid removed. February 8: fluid formed rapidly; patient became stuporous and irrational, later comatose; general condition poor. February 10: died.

Laboratory Findings.

Blood Counts

Date	Red blood cells (*millions*)	White blood cells
Dec. 19	4.8	8,400
Feb. 3	3.6	5,100
Feb. 6		8,100

Date	Chemical Examination of Blood		
	Icterus index	Urea nitrogen (*mg.* %)	Sugar (*mg.* %)
Dec. 19	188		
Jan. 22	148		
Feb. 6	140		
Feb. 7		22	104
Feb. 9	186		

Ascites. Ascites was common, occurring in about two-thirds of the cases. In many cases the day of appearance of ascites was known. The records stated that on one day the patient's abdomen was flat, and on the next it became distended. Thus, the onset of ascites was usually sudden. Ascites was a late manifestation; usually occurring only a few days before death (Table VII). There was one instance in which marked ascites developed relatively early, and after lasting several days disappeared.

TABLE VII

Interval Between Onset of Ascites and Death in 45 Cases of Epidemic Hepatitis

Interval (*days*)	No. of cases	Per cent of cases
1–4	22	49
5–9	10	22
10–14	3	7
15–19	4	9
20 and over	6	13

Case 100

Clinical Course. The patient was a white male, 27 years old. On June 12, appetite was poor; nausea but no vomiting; urine "dark"; itching at night. Some days later became severely nauseated but did not vomit; stools clay-colored; noticed that fatty foods particularly disagreed with him; slight pain in right upper quadrant. Admitted on June 22, with moderate jaundice; blood pressure, 130/60; abdomen flat; liver slightly tender, not enlarged. Condition remained about the same until June 28; patient was eating well; had no complaints; was ambulatory. July 4: jaundice deepened; still afebrile; went to mess hall and ate well. July 12: condition remained as stated. July 15: complained of not feeling well; jaundice of about same intensity; liver now painful and tender, not enlarged; no fever. July 20: had been vomiting; liver moderately enlarged; complained of pain in right upper quadrant; no abdominal fluid; no edema. July 21: nauseated; uncomfortable; pain in epigastrium; vomited occasionally; looked sick. July 22: abdominal fluid suspected; liver markedly tender; much pain in right upper quadrant; slight fever; seemed much worse; flanks slightly bulging. July 23: temperature, pulse and respiration were normal; abdomen more distended. July 24: slight rise in temperature; blood pressure, 120/65; abdominal fluid; suggestion of edema around ankles. July 25: paracentesis, 3500 cc. of bile-stained fluid removed; temperature, 101° F.; stools contained bile. July 26: condition considerably better; edema of ankles had cleared, but abdominal fluid had again accumulated. July 27: paracentesis, 3300 cc. of fluid withdrawn. July 28: continued to run low-grade fever; abdominal fluid recurrent; patient remained conscious and rational; liver slightly enlarged, but not tender. July 29: paracentesis, 2700 cc. of fluid withdrawn; liver still palpable, not

tender. July 30: patient in good spirits; felt better; temperature, pulse and respiration were normal; "ate three times today and for lunch had a lean steak." August 3: felt better, more alert, but had low-grade fever. August 5: paracentesis, 2300 cc. of fluid withdrawn. August 8: abdomen flat; no fluid; liver at costal margin; appetite still good. August 16: patient was not interested in food or in his surroundings; low-grade fever (temperature, 99.0° to 99.6° F.). August 21: no interest whatever; reflexes hypertonic. August 25: speech slurred and indistinct; response slow and frequently unintelligible; temperature, 100° F.; liver apparently had shrunken. August 28: bilateral ankle clonus, bilateral Babinski reflex; superficial reflexes hyperactive. August 31: stuporous. September 5: comatose; made smacking noises with lips; convulsive jerks of eyeballs; temperature, pulse and respiration were essentially normal. September 10: gradual rise in temperature to 104° F. on day of death. September 13: died.

Laboratory Findings.

	Urinalyses			
Date	Specific gravity	Albumin	Sugar	Bile
June 23	1020	o	None	+
July 21	1015	o	None	+
July 25	1013	o	None	
July 27	1015			
Aug. 19	1014	+	None	
Aug. 31	1004	++++	Trace	
Sept. 8	1028	Trace	++	
Sept. 11	1014	++++	+	
Sept. 13	1020	+++	+	

Chemical Examination of Blood

			Serum protein		
Date	Icterus index	Nonprotein nitrogen (*mg.* %)	Total (*gm.* %)	Albumin (*gm.* %)	Globulin (*gm.* %)
June 23	90				
July 21			5.28	2.79	2.49
July 23	116	43.5			
July 26	135	35			
July 30	92				
Aug. 7	50	35			
Aug. 17	57	36	8.24	6.68	1.56
Aug. 24	37				
Sept. 4	28				

Symptoms and Laboratory Findings

Analysis of symptoms and the results of laboratory examinations have been restricted to the following: temperature, pulse rate, blood pressure, examination of liver by palpation, recurrence of attack, hemorrhages, red blood count, leukocyte count, differential white count, plasma proteins and icterus index. In this analysis, the intermediate phase has arbitrarily been divided into two periods, namely, the first 3 days after the onset of jaundice, and the later period.

Temperature. An analysis of temperature is given in Table VIII. It will be seen that 92 per cent of the patients were afebrile during the first 3 days after the onset of jaundice. However, subsequent to this period 41 per cent developed fever. This usually was of brief duration. Since in many cases plasma, glucose, or whole blood was administered

intravenously, the febrile episodes cannot, with certainty, be attributed to hepatitis. Perhaps the most significant fact of the analysis is that approximately 60 per cent of the patients were afebrile throughout the intermediate period, but during the final period fever developed in the great majority, 89 per cent. Usually there was a sharp rise in temperature during the last 2 or 3 days of life when the patient was moribund (Text-Fig. 3).

Pulse Rate. In contrast to the bradycardia which is so common in other hepatic diseases, slowing of the pulse in this series was observed but seldom.

TABLE VIII
Temperature in Cases of Epidemic Hepatitis

	Number of cases		
	Intermediate period		Final period
	First 3 days after jaundice	Subsequent to first 3 days after jaundice	
Afebrile	24 (92%)	20 (59%)	6 (11%)
Febrile	2 (8%)	14 (41%)	49 (89%)
Total no. of cases	26	34	55

Blood Pressure. During the intermediate period the blood pressure was usually normal. With the onset of nervous manifestations, the pressure tended to rise. Representative examples follow: 144 systolic, 94 diastolic; 170/90; 100/80; 155/70; 150/92.

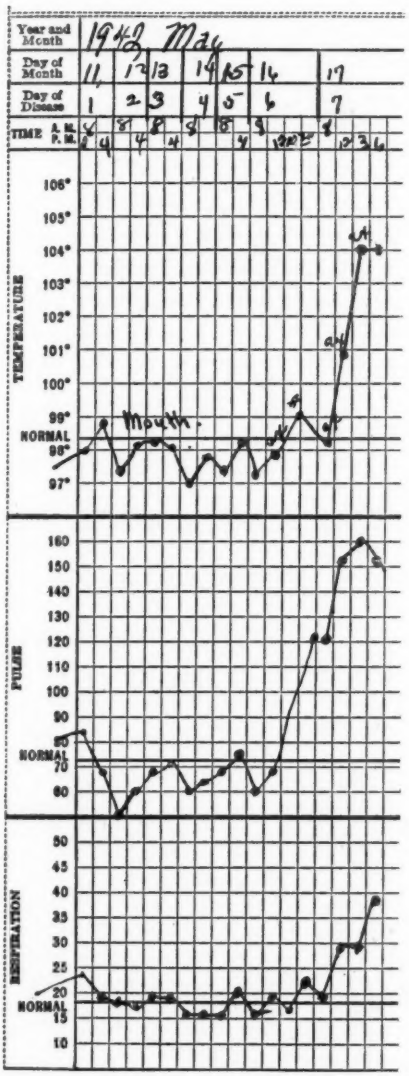
Palpation of Liver. During the first 3 days after the onset of jaundice, enlargement of the liver was found in approximately one-half the patients (15 of 28). Later, in the intermediate stage, enlargement was still more common (29 of 34 cases). Enlargement was usually associated with tenderness. During the final phase the majority showed shrinkage of the liver.

Recurrence. In only one instance did symptoms recur after an apparent recovery.

Case 117

Clinical Course. The patient was a white male, 22 years of age. In April, 1942, he noticed increased malaise and frequent nosebleeds. He later became jaundiced and was admitted to hospital, where he remained for 7 weeks. Course was uneventful except for daily rise in temperature, which usually reached a peak of 103° F. in the evening. Icterus index was as high as 113. His course, except for the fever, was similar to that of about 20 other patients with jaundice who were at that time hospitalized. June 8: transferred to another hospital; moderately ill, undernourished and jaundiced; liver enlarged and tender. Icterus index varied from 20 on admission to 120 on July 27, and then gradually fell to 11. Afebrile. Temperature, pulse and respiration had been normal since June 23. Steady improvement in general condition. September 14 to October 13: sick leave. October 27:

returned to duty. November 27: again became jaundiced. December 5: readmitted, apparently not acutely ill. December 11: ambulatory; did not feel ill, no fever. December 14: nausea and vomiting; jaundice had deepened; dull aching pain in right upper quadrant; stools had varied from clay-colored to light gray. December



Text-Figure 3

18: icterus index, 110; white blood cells, 4,500. December 20: icterus index, 150. December 21: vomited bile-stained material; stools brown. December 23: jaundice somewhat decreased; slight soreness in right upper quadrant. December 28: definitely worse; disoriented; later semicomatose and stuporous. December 29: died.

Hemorrhages. Hemorrhages were common, especially in the late period. Their incidence and distribution were as follows: petechiae of the skin in 20 instances, vomiting of blood in 20, epistaxis in 11 and other hemorrhagic phenomena (bleeding from bowel, hematuria and bleeding from respiratory tract) in 16 instances.

Blood Counts and Hemoglobin Determination. Representative examples of blood counts and hemoglobin determinations from 7 cases are given in Table IX. The differential white counts include only neutrophils and lymphocytes.

Erythrocyte Count. Erythrocyte counts in the different phases of hepatitis are summarized in Table X. During the first 3 days after

TABLE IX

Blood Counts in Representative Cases

Case no. and duration	Date	Red count	Hemoglobin	White count	Neutrophils	Lymphocytes
		(*million*)	(*per cent*)		(*per cent*)	(*per cent*)
No. 25. A.M.M.* no. 83171						
Onset, 5/29/42	6/6/42	4.26	75	7,500	64	32
Death, 6/27/42	6/26/42	3.88	70	4,550	57	34
No. 30. A.M.M. no. 83312	5/28/42	4.41	85	6,800	52	48
Onset, 5/24/42	6/19/42	4.00	80	5,200		
Death, 6/18/42						
No. 52. A.M.M. no. 83747	5/14/42	4.50	80	4,250		
Onset, 4/30/42	7/8/42	3.10	80	13,500		
Death, 9/15/42	7/15/42	3.80	80	9,100		
	8/2/42	2.75	55	10,800		
No. 59. A.M.M. no. 83833	7/7/42	4.65	78	8,000	50	41
Onset, 6/ 4/42	7/8/42	4.55	75	7,000	65	30
Death, 7/13/42	7/13/42	3.73		5,000	79	21
No. 84. A.M.M. no. 84422	6/16/42	4.6	80	3,600	51	49
Onset, 5/24/42	6/21/42	5.3	95	9,200	56	44
Death, 8/27/42	6/23/42			2,800	40	60
	7/2/42			5,600	50	50
	7/6/42	4.9	80	4,100	64	36
	7/29/42	4.3	85	4,400	63	37
	8/12/42	3.2	75	5,300	78	21
	8/14/42					
	8/18/42	2.9		4,900	68	30
	8/24/42	4.0	80	9,400	84	15
No. 86. A.M.M. no. 84506	6/21/42	4.50		6,700		
Onset, 5/25/42	7/7/42	3.25	80	4,200	67	33
Death, 7/26/42	7/16/42	3.00	75	5,800		
	7/19/42	2.20	65	5,600	63	37
	7/25/42	3.47	65	9,700		
No. 100. A.M.M. no. 85431	6/22/42	4.1	85	6,400	70	29
Onset, 6/12/42	7/20/42			6,200	63	33
Death, 9/13/42	7/23/42	3.8	87	5,500	62	38
	8/2/42	3.9		7,700	61	38
	8/11/42	3.2		9,000	65	32
	8/12/42			7,100	75	35
	8/16/42	4.3		9,500		
	8/24/42	3.1	73	7,700	74	21
	9/10/42			21,000	80	20
	9/10/42		76	22,000	83	17
	9/11/42	3.7		31,000	83	18

* A.M.M. = Army Medical Museum.

jaundice developed, the counts were approximately normal. Later the erythrocyte count fell. During the final phase definite anemia of secondary type was evident in approximately 40 per cent of the cases.

Leukocyte Count. The results of leukocyte counts are given in Table XI. Slight leukopenia was evident in nearly half of the cases early in

TABLE X

Erythrocyte Count in Cases of Epidemic Hepatitis

Erythrocyte count	Number of cases		
	Intermediate period		Final period
	During first 3 days after jaundice	Subsequent to first 3 days after jaundice	
(*millions*)			
Over 5	5 (22%)	9 (27%)	9 (24%)
4—4.9	18 (78%)	16 (48%)	14 (38%)
3—3.9	0	5 (15%)	10 (27%)	
2—2.9	0	3 (9%)	4 (12%)
Total number of cases	23	33	37

the intermediate phase. Leukocytosis during the intermediate period occurred in relatively few. The counts during the final period were strikingly different. Then few patients showed leukopenia, the majority having mild leukocytosis. This leukocytosis was probably related to terminal events, such as lobular pneumonia and phlegmonous inflammation of the gastrointestinal tract.

Differential White Blood Cell Counts. The results of differential

TABLE XI

Leukocyte Count in Cases of Epidemic Hepatitis

Leukocyte count	Number of cases		
	Intermediate period		Final period
	During first 3 days after jaundice	Subsequent to first 3 days after jaundice	
(*thousands*)			
Below 3.0	0	1 (2%)	1 (2%)
3.0—3.9	0	0	1 (2%)
4.0—4.9	5 (17%)	4 (10%)	4 (7%)
5.0—5.9	9 (30%)	9 (22%)	1 (2%)
6.0—6.9	2 (7%)	7 (17%)	5 (9%)
7.0—7.9	3 (10%)	9 (22%)	4 (7%)
8.0—8.9	6 (20%)	6 (15%)	6 (11%)
9.0—9.9	3 (10%)	1 (2%)	6 (11%)
10.0—10.9	1 (3%)	2 (5%)	7 (13%)
11.0—11.9	0	0	0
12.0—12.9	0	0	7 (13%)
13.0—13.9	1 (3%)	0	2 (4%)
14.0—14.9	0	1 (2%)	7 (13%)
15.0 and over	0	1 (2%)	5 (9%)
Total number of cases	30	41	56

counts are shown in Table XII. In this table the dotted horizontal lines mark the boundary between the normal and the abnormal. There was a relative lymphocytosis in two-thirds of the cases during the early days of the intermediate period. By contrast, during the final period a relative lymphocytosis was uncommon.

Plasma Proteins. In severe damage to the liver the plasma proteins would be expected to fall. In this series such a fall frequently did not

TABLE XII

Differential White Blood Cell Counts in Cases of Epidemic Hepatitis

	Number of cases		
	Intermediate period		Final period
	During first 3 days after jaundice	Subsequent to first 3 days after jaundice	
Polymorphonuclear leukocytes (*per cent*)			
40—49	0	2 (10%)	0
50—59	5 (28%)	6 (30%)	3 (12%)
60—69	10 (56%)	10 (50%)	2 (8%)
70—79	3 (17%)	2 (10%)	10 (38%)
80—89	0	0	8 (31%)
90 and over	0	0	3 (12%)
Total number of cases	18	20	26
Lymphocytes (*per cent*)			
50—59	0	2 (10%)	0
40—49	5 (28%)	7 (35%)	1 (4%)
30—39	7 (39%)	2 (10%)	4 (15%)
20—29	5 (28%)	8 (40%)	9 (35%)
Below 20	1 (6%)	1 (5%)	12 (46%)
Total number of cases	18	20	26

occur, probably because of therapeutic administration of plasma or whole blood. Representative examples are given in Table XIII.

Icterus Index. The icterus index in a number of representative cases, together with clinical abstracts, is given in Table XIV. The index is above normal. It fluctuates irregularly, but in the majority of cases it tends to rise. Exceptions to this are summarized in Table XV.

The icterus index during the three phases of hepatitis is shown in Table XVI. During the first 3 days the index was below 100 in the great majority of the cases. Subsequently it tended to rise, particularly so in the final period.

PATHOLOGIC ANATOMY

As has been stated, in every instance the fatal cases of epidemic hepatitis presented lesions in the liver that correspond to so-called idiopathic yellow or red atrophy. This condition has been described

in detail by numerous writers, and the literature on the subject has been exhaustively summarized by Roman [43] and by Herxheimer and Thölldte.[44] The paper by Wilson and Goodpasture [45] serves as an excellent introduction to the subject. It would be repetitious, therefore, to go into morphologic minutiae.

The changes observed will be presented in the following order: liver,

TABLE XIII

Plasma Proteins in Representative Cases
(gm. per 100 cc.)

Case no. and duration	Date	Total protein	Albumin	Globulin	Remarks
No. 52. A.M.M.* no. 83747	5/16/42	6.5	3.7	2.8	No ascites
Onset, 4/30/42	5/29/42	6.9	5.2	1.6	
Death, 9/15/42	6/ 6/42	7.7	3.0	3.9	
	6/15/42	6.7	4.4	3.3	
	6/29/42	5.5	3.1	2.9	
	7/15/42	5.0			
	7/20/42	5.4			
	7/29/42	6.7			
	8/ 5/42	7.2	4.9	2.3	
	8/10/42	7.6	5.0	2.6	
	8/17/42	7.3	5.7	1.5	
	8/24/42	9.2	6.5	2.4	
	8/31/42	7.2	4.7	2.5	
	9/ 7/42	7.2			
No. 84. A.M.M. no. 84422	7/ 8/42	5.26	3.31	1.95	Ascites since
Onset, 5/24/42	7/13/42	4.40	2.75	1.65	7/2/42;
Death, 8/28/42	7/16/42	4.9			repeated
	7/20/42	4.69	2.87	0.15	paracente-
	7/23/42	3.61	2.10	1.27	ses
	7/29/42	3.99	2.59	1.14	
	7/30/42	5.14	2.54	2.40	
	8/ 3/42	5.08	3.03	2.05	
	8/13/42	5.90	3.65	2.25	
No. 89. A.M.M. no. 84856	6/25/42	5.68			Ascites dur-
Onset, 6/1/42	7/ 4/42	6.6	4.3	2.3	ing last
Death, 8/18/42	7/20/42	6.65	3.7	2.6	few days
	8/10/42	4.1			of life
	8/15/42	3.4			

* A.M.M.=Army Medical Museum.

gallbladder, regional lymph nodes, ascites, spleen, gastrointestinal tract, hemorrhagic phenomena, bone marrow, kidney, testis, and brain.* No significant changes were found in the other organs.

Since no verbal description can give an adequate picture of the gross and microscopic changes of any disease, this paper is liberally documented by photographic illustrations.

* Major Philip Custer examined many sections of the bone marrow and spleen, and Captain Webb Haymaker those of the brain. This study has been greatly aided by their advice.

Liver

In all cases the liver is the site of the principal lesions and presents a characteristic picture. Without exception the changes are typical of idiopathic yellow atrophy.

Gross Appearance of the Liver

Grossly the organ is usually reduced, at times to less than one-half its normal size. Most often it weighs between 800 and 1200 gm. Reduction in weight, however, is not invariably found; in approximately one-fifth of the cases the weight of the liver falls within normal limits or is actually above the normal. Generally speaking, the smallest livers

TABLE XIV

Icterus Index in Representative Cases

Case no. and duration	Date	Icterus index	Clinical abstract
59 Onset, 6/4/42 Death, 7/13/42	6/13 6/17 6/24 6/29 7/1 7/7 7/10 7/11 7/13	36 78 143 127 136 177 210 190 204	White, male, 26 years old. 6/4: weakness, nausea, headache, anorexia, generalized aching, "dark" urine. 6/12: admitted; temperature, pulse and respiration normal; blood pressure, 114/80. 6/15: liver slightly enlarged. No change until 6/26, then abdominal pain; liver more enlarged and tender. 6/29: stuporous; occasional nausea and vomiting; apathetic. 7/3: jaundice increased. 7/8: blood pressure, 146/80; liver at costal edge. 7/10: blood pressure, 140/90; seemed less toxic; ate breakfast; responded to questions. 7/12: abdomen distended. 7/13: temperature, pulse and respiration began to rise; ascites noted; blood pressure, 140/78; temperature, 102° F. in morning, rose to 104° F. by afternoon; pulse, 130 to 150; respirations, 30 to 40.
57 Onset, 5/26/42 Death, 7/9/42	6/8 6/15 6/18 6/22 6/26 6/29	28 30 32 30 40 50	White, male, 20 years old. About 5/26: weakness, anorexia, nausea, headache, "dark" urine; later jaundice; said to have lost about 10 pounds; no itching. 6/8: admitted; ambulatory; not acutely ill; blood pressure, 104/68. Course in hospital stormy; persistent nausea and vomiting; deepening jaundice. 6/10: marked increase in size of liver to 4 fingersbreadth below costal margin; thereafter gradual decrease, by 6/30 to costal rim, during last week of life to 3 fingersbreadth above costal rim. 7/3: stuporous; comatose; spasmodic twitching of facial muscles; blood pressure, 84/50.
84 Onset, 5/24/42 Death, 8/28/42	6/20 6/29 7/6 7/8 7/10 7/18 7/20 7/24 7/27 7/30 8/7 8/10	160 160 150 150 150 100 90 60 60 45 45 35	White, male, 24 years old. About 5/24: anorexia, headache, backache, "dark" urine, clay-colored stools. 6/1: sclerae icteric. 6/4: admitted. Until 6/16, felt fairly well; then malaise, nausea, vomiting; liver tender, enlarged; "prognosis appears to be good." 7/2: ascites; repeated paracenteses. 7/23: jaundice declining; general condition improved. 8/10: appetite good. 8/13: greatly improved; condition satisfactory. 8/15: condition changed; became restless; dull; drowsy. 8/16: symptoms more marked; speech scanning; disoriented; progressive decline. During last several days ran irregular fever, temperature of 103° to 104° F.

TABLE XIV (*Continued*)
Icterus Index in Representative Cases

Case no. and duration	Date	Icterus index	Clinical abstract
23 Onset, 4/29/42 Death, 5/27/42	5/5 5/9 5/11 5/13 5/21 5/23 5/25	28 80 180 170 224 146 106	White, male, 26 years old. 4/29: nausea, vomiting, headache, constipation. 5/3: sclerae yellow. 5/5: admitted; temperature, pulse and respiration normal; liver not enlarged, slightly tender; blood pressure, 96/60; stools clay-colored. Remained in approximately same condition until 5/22, when jaundice became marked and vomiting began; liver markedly tender. 5/26: very restless; vomiting. 5/27: vomiting dark material containing blood; comatose.
52 Onset, 4/30/42 Death, 9/15/42	5/16 6/5 6/15 6/18 6/29 7/6 7/15 7/20 7/27 7/29 8/3 8/10 8/17 8/24 8/31 9/7	170 285 200 300 290 261 200 167 176 235 182 210 236 201 300 322	White, male, 27 years old. 4/30: nausea and anorexia. 5/2: "dark" urine. 5/4: jaundice; itching. 5/11: admitted; afebrile; liver just palpable. 6/17: said to have lost 21 pounds in 4 weeks; at times dull aching pains over region of gallbladder, thought to be due to obstruction of ducts. 6/22: exploratory operation; liver slightly enlarged, no gross abnormality; specimen taken for biopsy; gallbladder was small, wrinkled, almost empty, no stones; common duct was small, no stones. 7/15: edema of feet and ankles; marked itching. 8/3: itching continued; strength good. 8/10: patient continued to be up and about; felt well except for itching. 8/18: condition the same. 8/25: slightly weaker; irritable; appetite was less. 9/1: was gradually becoming weaker, walking only with crutches. 9/6: very weak, confined to bed; irrational at times. 9/14: comatose; weak; appeared to be dying from sheer exhaustion.

occur in patients who have died within 5 weeks. At later periods, weight loss tends to be replaced by compensatory hyperplasia of the remaining parenchyma.

The surface is usually smooth or finely wrinkled in the early case; at this stage its color is variable and not distinctive. Later, ivory colored or yellowish green coarse nodules, or larger, tumor-like masses project from the surface of the organ in some regions, whereas elsewhere the surface is sunken, dull red or grayish. The consistency, also, is variable. Usually flaccid in the early stages, the collapsed parts soon be-

TABLE XV
Trend of Icterus Index in 51 Cases of Epidemic Hepatitis

Trend		No. of cases	Per cent of cases
Remains below 50		6	12
Rises steadily		33	65
Rises, falls:			
(A) Rises, then falls slightly	9	11	22
(B) Rises, then falls to nearly normal	2		
Rises, then falls and afterwards rises again		1	2

come tough and meat-like. The nodular parts differ but little from the consistency of normal liver.

On the *cut surface* the irregular distribution of the lesions is even more conspicuous. There are large, red, meat-like areas which ooze blood, and in which the landmarks are indistinct or obliterated. In sharp contrast to these red areas are irregularly distributed patches of pale yellow or bile-tinted tissue which are distinctly lobulated. These yellow patches range in size from small nodules to large confluent masses which may occupy the major part of the organ. The component

TABLE XVI

Icterus Index in Cases of Epidemic Hepatitis

Icterus index	Number of cases		
	Intermediate period		Final period
	During first 3 days after jaundice	Subsequent to first 3 days after jaundice	
Below 50	8 (35%)	2 (3%)	4 (8%)
50–99	12 (52%)	12 (20%)	7 (13%)
100–149	2 (9%)	18 (30%)	6 (11%)
150–199	1 (4%)	16 (27%)	9 (17%)
200–249	0	7 (12%)	10 (19%)
250–299	0	2 (3%)	9 (17%)
300–349	0	1 (2%)	5 (10%)
350–399	0	1 (2%)	2 (4%)
400 and over	0	1 (2%)	1 (3%)
Total	23	60	53

lobules are abnormally large and vary greatly in size and shape. This tissue is ischemic. Usually it has a "healthy" appearance; it is not fatty nor turbid.

The relation of size of the liver to duration of the disease is given in a graph (Text-Fig. 4), and the data from which the graph was constructed are presented in Table XVII. Disregarding for the present the association with ascites, it will be seen that marked reduction in size generally occurs when the duration of the disease is short, whereas large livers are more often found when the course has been protracted.

Irrespective of the duration of the disease, the weights of the liver show considerable scattering; they range from 600 to 2400 gm. In approximately one-fifth of the cases, the weight of the liver is normal or above normal (taking 1400 to 1600 gm. as the average normal weight). In four-fifths of the cases the liver is smaller than normal. This reduction is extreme in 10 livers, which weigh from 600 to 800 gm. Hence, the liver in yellow atrophy usually is shrunken, but occasionally is enlarged.

The variability in size is equalled by the variability in shape, color, consistency and appearance of the cut surface. This variability is

TABLE XVII

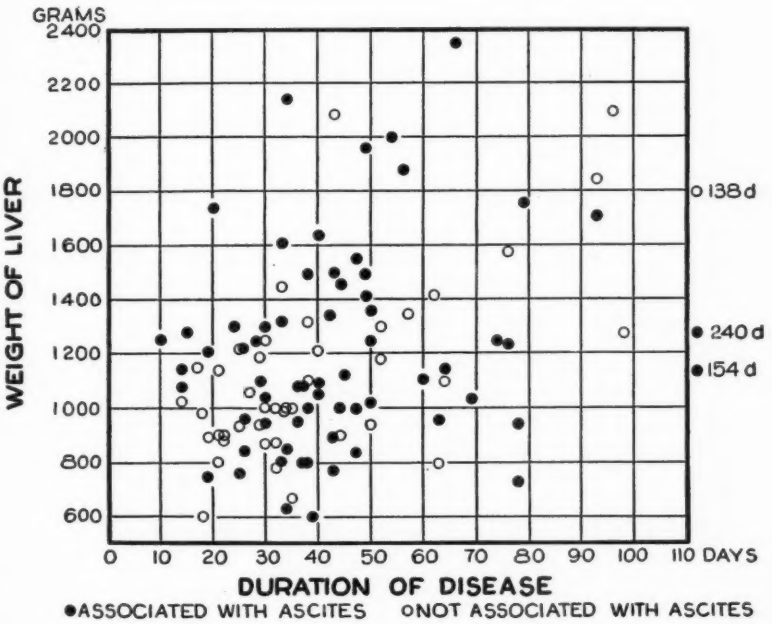
Relation of Duration of Disease to Size of Liver and Occurrence of Ascites in 108 Cases of Epidemic Hepatitis

Duration of disease	Weight of liver (gm.)												Total		Grand total
	600–799		800–999		1000–1199		1200–1399		1400–1599		1600 and over				
	Ascites	No ascites	Ascites	No ascites	Ascites	No ascites	Ascites	No ascites	Ascites	No ascites	Ascites	No ascites	Ascites	No ascites	
(*days*) 10–19	1	1		2	2	2	3	0					6	5	11
20–29	1		2	6	1	3	3	1			1		8	10	18
30–39	2	2	6	2	4	6	2	2	1	1	2		17	13	30
40–49	1		2	1	5		1	1	5		2	1	16	3	19
50–59				1	1	1	1	2			3		5	4	9
60 and over	2		1	1	4	1	2	1		2	4	3	13	8	21
Total	7	3	11	13	17	13	12	7	6	3	12	4	65	43	108
Grand total	10		24		30		19		9		16		108		

characteristic of epidemic hepatitis; the liver is not damaged uniformly, but the extent of damage in different areas varies widely. Large masses of parenchyma are destroyed completely, whereas elsewhere damage is moderate.

The gross changes found may now be briefly described in representative cases.

The External Appearance of the Liver. The liver shown in Figure 1 represents the external appearance seen in the majority of cases. The



Text-Figure 4

duration of hepatitis was 36 days. The liver weighed 1320 gm. The left lobe is seen to be disproportionately shrunken. The surface is uneven and varies in color. Over approximately half of the right lobe, the tissue protrudes in the form of irregularly elevated, yellow nodules. Elsewhere, the surface is sunken, gray-red, and the capsule is smooth or finely wrinkled. In the left lobe the surface is deeply furrowed. When fresh the consistency of this lobe was meat-like; by contrast, the yellowish elevations of the right lobe felt approximately like normal liver.

A much greater shrinkage and deformity of the liver is shown in Figure 10, although in this case the duration of hepatitis was prac-

tically the same as in the case from which the preceding one was obtained, namely, 37 days. The organ weighed 800 gm. Large, yellowish green bosses project from the surface. Between these, the surface is grayish red, smooth, or finely wrinkled. Thus, in two cases of practically the same duration, the livers appear very different.

An even greater deviation is shown in Figure 9. The duration of hepatitis was 69 days. The liver weighed 1040 gm. Many large, greenish masses project like tumors above sunken, reddish patches, the surface of which has the appearance of coarsely grained leather. In gross appearance this liver is reminiscent of hepar lobatum.

The *cut surface* of the liver in one of the early cases of the series is shown in Figure 2. The duration of hepatitis was 19 days, and the weight of the liver was 890 gm. In the right lobe are large confluent nodular areas of ivory color. They are notably ischemic. Lobulation is distinct; most of the individual lobules are conspicuously large. In contrast to the ischemic yellow areas the cut surface of the rest of the liver is uniformly reddish brown. Because of the color, there is a superficial resemblance to liver parenchyma, but there is no lobular pattern.

Little further change in the appearance of the cut surface is shown at a later stage of hepatitis, 43 days in duration (Fig. 3). The organ weighed 850 gm. Two contrasting regions are seen, one a yellow-green nodular mass, the other, a reddish brown, meat-like tissue.

In the four examples given, the livers were shrunken. Enlarged livers are shown in Figures 11 and 12. Fig. 11 illustrates the cut surface of a liver which weighed 1710 gm. The duration of hepatitis was 93 days. Most of the tissue consists of strikingly large, pale lobules. They bulge above sunken, smooth tissue which, when fresh, was gray-red and firm.

Another enlarged liver (2100 gm.) is shown in Figure 12. The duration of the hepatitis was 96 days, *i.e.*, practically the same as in the preceding case. The entire right lobe is uniformly composed of irregular lobules having pale peripheries and dark centers. The left lobe is small and gray.

Microscopic Appearance

Parenchymal Destruction. In sections from the red areas, the liver cells have disappeared completely, but the lobules are still outlined by small proliferating bile ducts. The fact that, despite complete destruction of the parenchyma, the outlines of the lobules may still be recognized is highly characteristic of epidemic hepatitis. A low-power view of this appearance is shown in Figure 17. It is seen that, because of the disappearance of liver cells, the portal triads lie nearer together and that lobular outlines are indicated by small bile ducts. Greater

detail is shown in Figure 18, where numerous small bile ducts appear to form a fence around the lobules.

The *sinusoids* in areas of "red atrophy" are preserved and are often greatly engorged (Fig. 4). Sometimes, however, the sinusoids are collapsed, and grossly such areas appear gray.

The reticular *framework* of the lobules is not destroyed. Its meshes are narrowed, or even collapsed, and its fibers thickened. The preservation of the reticulum is shown in a case of average duration, 30 days, and in one of prolonged duration, 96 days (Figs. 19 and 20). Through this framework are scattered numerous lymphocytes, plasma cells, granulocytes and macrophages; their relative proportions vary from case to case, and with the duration of the disease. Thus, as the result of a destructive process, entire lobules throughout large areas of the liver are reduced to their skeletal frames. Here, an inflammatory reaction is evident; that is, a hepatitis.

Absence of Scarring in Areas of Destruction. Although the reticulum fibers may become densely compressed, there is little or no formation of collagen. Scarring such as occurs in cirrhosis and accompanies the healing of abscesses or gummas is characteristically absent in epidemic hepatitis.

Rapid Disappearance of Liver Cells. The sequence of events that have led to the emptying of the lobules cannot be traced with certainty. None of the livers in this series were in the early stages of destruction; indeed, so far as I have been able to learn from the literature, no one has ever seen the earliest stages in this disease, which rarely terminates in its most acute stages. All that is known with certainty is that cell destruction occurs rapidly and that cell débris is removed speedily. In the present series, by the tenth day (the earliest case of the series) practically all traces of dead cells have been swept away, presumably by enzymic action, and nothing remains in the lobules but vascular framework and inflammatory cells. We find here no evidence of slow cell death—fatty changes, coagulation of cytoplasm—such as are characteristically seen in yellow atrophies due to chemical poisons, bacterial toxins, yellow fever, or eclampsia. Rapidity and completeness of cell destruction is a distinguishing feature of epidemic hepatitis.

Distribution of Lesions in the Lobule. The destructive change usually, if not always, begins in the central part of the lobules, for in the earliest cases of the series only the central zones of the lobules are involved (Fig. 13). Even in more protracted cases many lobules are encountered in which the damage is limited to the central regions, leaving intact a peripheral rim of liver tissue (Fig. 14). The lesions do not involve all lobules to the same degree; they range from destruction of

approximately one-third of the central portions in the better preserved areas to complete destruction of all liver cells in large areas of the organ. Every conceivable intermediate step may be encountered. But whenever even a fragment of hepatic parenchyma remains, it is found in the peripheral zone. For example, in another early case, only scattered groups of liver cells are left at the lobular peripheries (Fig. 15).

Inflammatory Reaction. In epidemic hepatitis destruction of liver cells is invariably accompanied by an inflammatory reaction. By inspection of Figures 13 to 15, which show zones of destruction, it may be seen that the parts of the lobules from which the parenchyma has disappeared have a granular aspect. This granularity is due not to remaining débris but to the presence of inflammatory cells. In early stages polymorphonuclear leukocytes, lymphocytes, plasma cells and macrophages occur in approximately equal proportions (Fig. 16); later, the polymorphonuclear leukocytes become less numerous. The inflammatory cells linger in the depleted stroma for long periods, only gradually becoming less numerous. For example, in a case with a course of 240 days, the inflammatory changes did not differ appreciably from those in the average case of a few weeks' duration.

Lipofuscin. Conspicuous among the inflammatory cells are macrophages which have engulfed small granules of yellow-brown pigment (Fig. 6 a). This pigment is not dissolved in the process of preparing paraffin sections; in such sections it is stained by Sudan III and similar fat stains (Fig. 6 b). It is somewhat acid-fast, and can be demonstrated by the Ziehl-Neelsen method.

Silver stains, such as Wilder's reticulum stain, render the granules black. The pigment is lipofuscin, or so-called "waste-pigment."[46, 47] Lipofuscin is probably a normal pigment of liver cells which is liberated and rapidly phagocytized when these cells disintegrate. It thus serves as an indicator of breakdown of hepatic parenchyma. Like the other types of cells previously discussed, the pigmented macrophages may persist for long periods.

Lipofuscin must be distinguished from another pigment that may also occur in the liver and which in recent years has attracted considerable attention, namely, ceroid. The main points of difference are that ceroid forms coarse globules that often lie extracellularly, it is strongly acid-fast and even less soluble in fat solvents than lipofuscin, and in paraffin it stains deeply with fat stains.[48]

Endophlebitis of Efferent Blood Vessels. The efferent vessels show marked alterations. All but the largest hepatic veins are the site of endophlebitis. The process is seen particularly in the central lobular veins and in the sublobular veins (Figs. 21 and 22). In these vessels

the intima is densely infiltrated with cells of the same types as those scattered throughout the skeletal remnants of the lobules. Where inflammatory cells are abundant in the stroma, they are usually abundant in the intima of veins that drain the area. In early stages the endothelium of the intima is unbroken; in later stages it may become ruptured, spilling the invading cells into the vascular lumen. Rupture of the intima is commonly followed by thrombosis.

Not only the intima but other layers of the vessels are altered. Often their walls are greatly thickened, and have a hyaline appearance (Fig. 21). They usually stain rather lightly with eosin, but densely by the Masson-Mallory aniline-blue method for connective tissue. By silver impregnation methods, the walls of the veins are found to have a loose, fibrillar structure, which differs but little from the normal (Fig. 23). It is evident that the collagenous substance, which gives the hyaline appearance to the wall, lies between the component fibers, which themselves remain unchanged. Perhaps this collagenous substance becomes more prominent through imbibition of fluid.

Endophlebitis usually is more conspicuous in relatively early cases, although lesser degrees are found even in protracted cases. In the latter group, however, fibrous obliteration of the veins rather than acute inflammatory reaction is the more common.

This form of endophlebitis is not specific for the damaged liver of epidemic hepatitis. It may occur in other destructive processes involving liver parenchyma.

Hyperplasia of Liver Cells

The preceding sections have dealt with the microscopic appearance of the liver in areas which grossly are collapsed and red; here, the parenchyma has been destroyed. In contrast, in areas which appear nodular and yellow, the tissue is in a state of regenerative hyperplasia. An abundance of new parenchyma has formed by hypertrophy and multiplication of cells that have escaped destructon. The architectural pattern of the new parenchyma, however, only rarely approaches the normal; in most regions it is exceedingly atypical. The process of restoration begins early. By the tenth day numerous buds of binucleated or multinucleated liver cells extend from the remaining portions of the hepatic columns into the depleted stroma (Figs. 24 to 26). In less than 20 days a large mass of new liver tissue has formed. This tissue usually is composed of atypically built "lobules." They vary in size and shape. Most of them have fused with neighboring "lobules"; less often patches of parenchyma are isolated by bands of collapsed stroma.

The microscopic appearance of such a region in one of the earlier cases (19 days in duration) is illustrated in Figure 31. The section comes from the pale yellow part of the liver shown in a colored photograph (Fig. 2). Inspection of the photomicrograph discloses at once the absence of the lobular pattern characteristic of normal liver. Instead, the parenchyma consists of ill-defined patches, in none of which the component cords of cells converge toward a common central lobular vein. These patches represent hyperplastic remnants of former lobules.

An illustration of the atypical structural arrangement of the newly formed tissue in a case of prolonged duration (93 days) is given in Figure 32. There are seen great confluent patches of parenchyma but no typical lobules.

These two illustrations represent the microscopic picture observed in nearly every case. Only occasionally, and then in small areas only, is normal lobulation found restored (Fig. 33).

The hyperplastic parenchyma is composed chiefly of compact cords of liver cells, but often such cords have formed only at the periphery of the individual "lobules." The central part is frequently occupied by newly formed cells which are not organized into cords (Fig. 34).

Areas of regeneration are usually ischemic (Fig. 5). Well defined spaces, with endothelial lining, lie between the cell cords; but the blood content of the spaces is usually slight, and only in rare instances approaches the normal. The marked degree of ischemia of hyperplastic tissue stands in contrast to the great engorgement of the tissue in areas of "red atrophy" (Fig. 4).

The hyperplastic tissue differs from the normal not only in general arrangement but in structure of its component parts. The cords of liver cells are usually distinctly broader than the normal. In longitudinal sections the cords often have a conspicuous central cleft. In transverse sections the cords are seen to have a tubular structure; such tubules have from six to eight cells spaced around a lumen (Fig. 37). The clefts or lumina are dilated bile canaliculi. Many are obstructed with coarse branching masses of bile (Figs. 37 and 38). Throughout large areas, almost every canaliculus contains obstructing masses.

The individual liver cells generally are large. In some the cytoplasm is smooth; in others it contains coarse granules of bile. Fatty changes in the cells are rare. The nuclei are usually prominent, and the nucleoli are swollen and stain deeply (Fig. 36). Inclusion bodies, such as occur in many virus diseases, are not found.*

* I am grateful to Dr. Thomas Rivers of the Rockefeller Institute, to Dr. E. V. Cowdry of the Washington University School of Medicine, and to Dr. Alfred M. Lucas of Iowa State College for examining a number of my sections and for their helpful advice.

The foregoing paragraphs have dealt with the appearance of tissue in which the hepatic cells have formed cords. Here and there, however, the regenerating cells do not become arranged in columns but lie isolated or in small groups within an otherwise empty stroma. Such cells tend to form multinucleated syncytia (Figs. 27 and 28), or disorderly groups which invade the stroma in a manner faintly suggestive of neoplasia (Fig. 29). Still others form giant tubules reminiscent of those seen at early developmental stages or in the livers of frogs or turtles (Fig. 5).

In most areas the cells of the hyperplastic parenchyma, whether arranged as cords or in isolated groups, have a healthy appearance. There is no evidence of progressive destruction of tissue. But in a few areas the regenerated cells may undergo secondary degeneration, as shown in Figure 35. Here there is a large group of newly formed cells with prominent nuclei which obviously are disintegrating. The tissue is invaded by numerous polymorphonuclear leukocytes.

Among the factors that lead to such secondary destruction are probably ischemia and the accumulation of metabolic products, which are not removed because of stasis of blood and of bile.

Proliferative Changes in the Bile Ducts

Besides regenerative phenomena in the liver cells there are proliferative changes in the bile ducts. Before discussing them it is well to recall that a sharp distinction should be made between interlobular and perilobular (septal) bile ducts.[49] The former are relatively large, and, together with branches of the portal vein and hepatic artery, lie within a thick mantle of connective tissue. The lining epithelium of these ducts is prominent. At the outskirts of the stroma which enfolds the portal triads, and extending around the lobular peripheries, lie the much smaller septal biliary ducts. Unlike the large interlobular ducts, the smallest septal ducts have a very scanty connective tissue stroma. They are tributaries of the interlobular ducts, and they accompany the fine twigs of the portal veins and hepatic arteries which encircle the lobular peripheries. In the smallest of these minute ducts the epithelium is inconspicuous and so flat that it resembles the lining of the vessels. Even in the larger branches the cells are relatively small and hence the nuclei appear crowded. The cytoplasm stains rather lightly in contrast to the deeply eosin-staining cytoplasm of liver cells; the nuclei are round or oval, and much denser than those of hepatic cells. Into these ducts empty the bile canaliculi of the hepatic cords; the junction usually is expanded into an ampulla.

The large interlobular ducts show no changes in epidemic hepatitis. Their cells remain normal and their lumina patent (Fig. 39). On the contrary, the small septal ducts do proliferate markedly. This is shown in Figure 40. (The duration of the disease in this case was 14 days.) At the periphery of the portal stroma are numerous small bile ducts; all of them show budding. The ducts and their buds soon elongate, forming extensively branching tubular structures (Fig. 41). Often their lumina contain inflammatory cells, similar to those within the stroma of the lobular remnants. Ducts of this kind outline the periphery of former lobules (Figs. 17, 18 and 30).

The fate of these proliferated ducts has long been a subject of discussion. It is certain that in many instances they remain entirely unchanged for long periods. Thus, Figure 42 shows the appearance of an area of "red atrophy" from a case of 240 days' duration. The ducts here do not seem much different from those shown in Figure 40, a case of 14 days' duration. In this and similar regions from other cases which have had a protracted course, there is no indication that the ducts tend to differentiate into other types of cells.

Conditions, however, are not always as simple. There are found in early, as well as in late, cases, at the periphery of former lobules, tubular structures which cannot with certainty be recognized as bile ducts. For example, in Figure 43 there are seen several tubules, some parts of which are lined by cells resembling bile duct epithelium, whereas other parts are lined by elements resembling liver cells. More complicated still is the interpretation of pictures such as that shown in Figure 8. Here the lumen of a branching bile duct is continuous with that of a tubule composed of liver cells. Surrounded by the epithelium of the bile duct lie several large and typical liver cells. This appearance suggests a possible transformation of biliary epithelium into hepatic epithelium. Equally complicated is the interpretation of the condition shown in Figure 44. Here are seen two large clumps of inspissated bile. One clump lies within a dilated tubule, the lining cells of which resemble biliary epithelium. The other clump lies within a dilated lumen of a hepatic cord which on one side is composed of biliary epithelium. Similarly, in Figure 45 are shown a number of tubules at the periphery of lobular remnants; the lumina of these tubules contain thick clumps of bile; the cells which compose these tubules cannot with certainty be identified either as biliary epithelium or hepatic epithelium. Still another puzzling condition is shown in Figure 46. In this case the tubules at the periphery of the lobules are composed of cells which closely resemble liver cells; and, similarly, in Figure 47 there is a whole cluster of tubules which appear to be made up of hepatic epithelium. Lastly,

there is shown in Figure 7 a not uncommon finding. Here a branching bile duct is in contact with two large hyperplastic hepatic columns; the lumen of the duct clearly is continuous with the lumina of these columns.

Pictures such as have been shown raise again the long debated question: is biliary epithelium transformed into liver cells? This question has been debated for many years. Pearce [50] wrote in 1906: "Some difference of opinion exists in regard to the solid and tubular cell-strands usually described as newly-formed or pseudo bile ducts. All recent investigators have abandoned the idea that these structures are atrophied rows of liver cells, but consider them as sprouts from the small interlobular bile ducts growing into the necrotic lobule. By a gradual transformation of the cells of such sprouts true functioning liver cells are formed."

Pearce [50] expressed well the debated problem. In recent years Herxheimer and Thölldte,[44] and others again have taken the opposite view, namely, that the structures so characteristically seen at lobular peripheries are not bile ducts but altered liver cells. This opinion is based largely upon the fact that within these structures bile canaliculi or masses of bile may be demonstrated. It might be suggested, however, that, when much of the liver parenchyma has been destroyed, the cells of proliferating bile ducts may acquire a compensatory capacity to metabolize bile.

The question cannot possibly be settled by examination of post-mortem material. In my opinion, biliary epithelium may differentiate into hepatic epithelium. Such differentiation, however, occurs only in a few insignificant patches and contributes very little to the restoration of tissue which at best is atypical.

Gallbladder and Extrahepatic Bile Ducts

The condition of the gallbladder was recorded in 87 cases; in 36 it was described as normal, in 30 as small, and in 21 as distended. In about half the cases the wall was edematous and occasionally showed small hemorrhages (Fig. 55). In these cases ascites was almost invariably present.

The extrahepatic ducts were carefully examined in nearly all cases, with particular attention to their patency. In only two instances was obstruction found. In these two, small plugs of inspissated material occurred in the common duct near the duodenal opening. It is probable that in both instances the obstruction was a sequel to the hepatic lesion, not its cause.

Material for microscopic examination from the extrahepatic ducts

and the papilla of Vater was available in only 11 cases. In 6 there was inflammatory edema, always associated with edema of the duodenum. The duodenal inflammation was not a localized condition but widespread, involving at times almost the entire gastrointestinal tract.

Lymph Nodes

In nearly all cases, particularly in those in which the duration of hepatitis was less than 1 month, the hepatic, and in many instances the mesenteric, lymph nodes were considerably swollen, their capsules tense, and their cut surfaces bulging and succulent (Fig. 1). Occasionally individual nodes measured as much as 35 mm. in diameter. Microscopically, the sinusoids were dilated and frequently packed with polymorphonuclear cells and macrophages. The reticulo-endothelial lining was prominent. The lymphoid tissue was markedly edematous. In occasional nodes, small foci of necrosis and hemorrhages were encountered. Briefly, the changes in the regional lymph nodes were edema, acute hyperplasia, or acute lymphadenitis, *i.e.*, the usual reactions found near areas of tissue destruction. Involvement of lymph nodes was never generalized.

Ascites

Ascites occurred in 60 per cent of the cases. The amount of fluid usually was large, two or more liters being the average. The fluid was nearly always tinged with bile, less often with blood. Turbidity due to the presence of inflammatory cells was common when phlegmonous inflammation of the gut coexisted (this condition will be discussed later).

The relation between ascites, duration of disease and size of liver is shown in a distribution graph (Text-Fig. 4) and in Table XVII. The weight of the liver has been plotted against duration of disease; cases with ascites are represented by solid circles, those without ascites by open circles. The graph brings out several points. First it shows that ascites may occur at all stages of the disease; no matter whether the course is short or protracted. The occurrence of ascites is not definitely correlated with duration of disease (see Table XVII).

Regarding the relation of size of liver to occurrence of ascites, there is again no apparent correlation. Thus, the incidence of ascites when the liver was greatly shrunken (below 800 gm.) is about the same as when it was enlarged (above 1600 gm.).

In approximately one-fourth of the cases with ascites there was pleural effusion; and in approximately one-half the cases, slight edema of the ankles. The clinical records indicate that ascites precedes ankle

edema, which was probably due to mechanical interference with venous return.

In Bergstrand's[41] series of 95 fatal cases, ascites was observed in 20 per cent. Fox and his co-workers[42] found ascites in most of their 17 cases.

In summary, it is shown that ascites is common and that it is unrelated to the total duration of the hepatitis or to the size of the liver. The mechanism responsible for the accumulation of abdominal fluid will be discussed later.

Spleen

The spleen was enlarged in about three-fourths of the cases. The weights are given in Table XVIII. In one-third of the series the weight exceeds 300 gm.; in somewhat less than half the weight falls between

TABLE XVIII

Relation of Weight of Spleen to Occurrence of Ascites in 89 Cases of Epidemic Hepatitis

Weight of spleen	Number of cases		
	Ascites	No ascites	Total
(*gm.*)			
Below 100	0	1 (1%)	1 (1%)
100—199	10 (11%)	11 (12%)	21 (24%)
200—299	23 (26%)	15 (17%)	38 (43%)
300—399	15 (17%)	6 (7%)	21 (24%)
400—499	2 (3%)	1 (1%)	3 (3%)
500 and over	4 (5%)	1 (1%)	5 (6%)
Total	54 (61%)	35 (39%)	89

200 and 300 gm., in only one-fourth of the cases is the weight approximately normal.

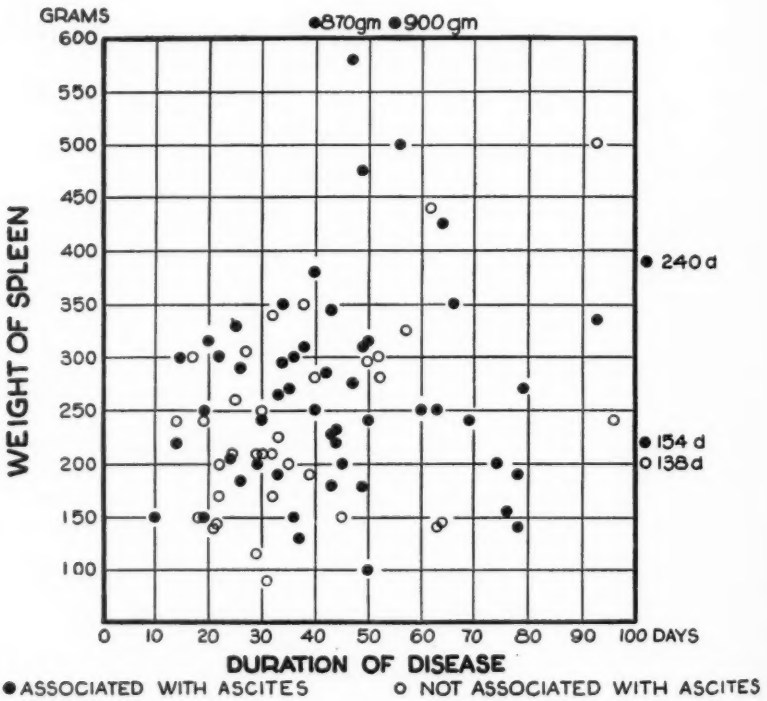
The relation of weight of spleen to duration of hepatitis is shown in Text-Figure 5. There is much scattering but, generally speaking, large spleens, above 300 gm., are more numerous when the disease has been prolonged (see also Table XVIII).

In consistency, the spleen in early cases was flaccid, in later cases, firm. On the cut surface the lymphoid follicles were usually distinct at early stages, and small or indistinct later. At all stages, the blood content of the spleen was increased.

The microscopic appearance also varied with the duration. In relatively early cases, the lymphoid tissue was prominent (Fig. 56); the sinusoidal veins and the pulp cords were only moderately congested. At later stages, the sinusoids were conspicuously distended and had thickened rigid walls (Fig. 57). The lymphoid tissue was often markedly depleted (Fig. 58). The picture then was that usually associated with portal hypertension.

Correlating the gross and microscopic findings, it becomes evident that the enlargement of the spleen in early stages is not the result of engorgement but is predominantly due to a cellular proliferation. At later stages sinusoids become dilated; then swelling is due to engorgement.

The early lymphoid hyperplasia is probably a reaction to products of tissue breakdown in the liver. It cannot be denied, however, that the agent which damages the liver may itself excite a reactive process in the spleen. The depletion of lymphoid tissue, during later stages of hepatitis, may result from portal hypertension, that is, from overload-



Text-Figure 5

ing of the spleen with venous blood under increased pressure.

This interpretation is supported by recent experimental studies of British investigators. Thus Orr [51] studied the spleens of rats, the livers of which had been damaged by "butter-yellow" (a substance which induces a cancerous cirrhosis of the liver). Enlargement of the spleen developed early, long before the liver became cirrhotic. Orr favors

the view that such early enlargement of the spleen is due to cellular hyperplasia. Menon [52] induced hepatic destruction and late cirrhotic changes by carbon tetrachloride. He found a marked proliferative reaction in the spleen during the early stages of liver damage. Similarly, experiments of Cameron and de Saram [53] suggest that splenic changes associated with cirrhosis of the liver (a condition of progressive damage) are the result of two separate processes, namely, pulp hyperplasia and congestion.

Gastrointestinal Tract

Lesions of several kinds and of great interest were encountered in the gastrointestinal tract. The most striking of these was an extensive phlegmonous inflammation with massive edema involving the ileocecal region, less commonly other portions. In addition, noninflammatory edema of both the small and large intestines was frequent, and the lower end of the esophagus was often ulcerated.

Phlegmonous Inflammation

In approximately 15 per cent of the cases in which the gastrointestinal tract was examined (17 of 113), large parts of the gut were phlegmonous. The lesion was usually most marked in the ileocecal region, but sometimes extended more widely, involving the ascending and transverse colon. The wall of the cecum sometimes exceeded 20 mm. in thickness. It was extremely boggy, and its mucosa was thrown into thick folds. The wall of the gut appeared as if artificially injected with a watery fluid (Figs. 48 and 49). Microscopically (Figs. 50 and 51), the entire wall was edematous and everywhere invaded by leukocytes and macrophages. Usually the mucosa was unbroken. Occasionally, superficial hemorrhages were found, but actual erosions were rare. The submucosal layer was especially distended, and even in paraffin sections (in the preparation of which edematous tissue shrinks) exceeded 1 cm. in thickness. The fibers of the mucosa were forced apart, and in some foci appeared to be degenerating. Also the subserosal layer was often considerably distended and invaded by inflammatory cells.

Most of the infiltrating cells had well stained nuclei and intact cytoplasm; they were obviously recent invaders (Fig. 52). The proportion of the cells varied; in some regions polymorphonuclear leukocytes, and in others macrophages, predominated, while lymphocytes and plasma cells were scanty. With stains for bacteria an abundance of small Gram-negative bacteria and larger Gram-positive rods were seen. Coccoid forms were not found. Many of the leukocytes and macro-

phages had ingested these organisms, and some macrophages, particularly, were packed with bacteria.

In nearly all cases, other parts of the small and large intestines were conspicuously edematous, and in some areas the distended tissue showed early inflammatory reaction. In only two cases was a phlegmon found in the stomach.

Without exception, the phlegmonous inflammation was associated with ascites. Careful examination of the clinical records gave no hint of the intestinal lesion. The duration of the phlegmon was always short, as shown by the condition of the exudate; it was evidently a terminal inflammation.

What is the relation between hepatitis and the inflammation of the intestine? The phlegmon gives every appearance of being a terminal event. Therefore it is obvious that the hepatitis is not the result of the intestinal condition. Rather, the phlegmon is a complication which results from invasion by bacteria of an edematous bowel.

In his monograph on yellow atrophy of the liver, Bergstrand [41] merely mentions phlegmonous inflammation of the intestine in several of his autopsy protocols. Recently, Pollack and Gerber [54] have reported a number of cases, in all of which the lesion was associated with primary disease of the liver. I have been able to find no other references in the literature.

Because intestinal phlegmon is a little known condition, brief abstracts of the anatomic findings in the 17 cases are given below.

Illustrative Cases

Case 8

This patient was a white male, 28 years old. Duration of hepatitis, 43 days. (See page 482 for clinical course.)

Pathologic Findings. Esophagus: (G)* superficial ulceration at cardio-esophageal junction; (M)* ulceration; inflammatory reaction extending to muscularis. *Stomach:* (G and M) normal. *Intestine:* (G) marked edema beginning just above ileocecal valve and extending to sigmoid; moderate edema of remainder of small and large intestine. *Duodenum and papilla of Vater:* (M) mucosa normal, intense congestion; submucosa invaded by numerous polymorphonuclear leukocytes and histiocytes. *Cecum:* (M) mucosa intact; wall markedly edematous, dense leukocytic and histiocytic infiltration throughout entire wall; fibrinocellular exudate on serosa.

Case 10

The patient was a white male, 22 years of age. Duration of hepatitis, 30 days.

Pathologic Findings. Stomach: (G) mucosa congested; pylorus markedly edematous; (M) marked edema of submucosa. *Duodenum and papilla of Vater:* (G)

* The letter (G) signifies results of gross examination, and (M), of microscopic examination.

extremely marked edema; (M) marked edema; slight leukocytic infiltration; subserous hemorrhages. *Jejunum and ileum:* (G) hemorrhagic; (M) submucosa and subserosa markedly edematous; slightly infiltrated with leukocytes. *Large intestine:* (G) ascending and transverse colon hemorrhagic; (M) mucosa intact; entire wall markedly edematous and heavily infiltrated with polymorphonuclear leukocytes and histiocytes; phlegmon most pronounced in submucosa; serosa showed no reaction.

Case 11

This patient was a white male, 41 years old. Duration of hepatitis, 64 days.

Pathologic Findings. Esophagus: (G) congestion at lower end. *Stomach:* (G and M) multiple petechiae of mucosa. *Small intestine:* (G) moderately edematous; terminal portion of ileum markedly edematous; (M) marked polymorphonuclear, neutrophilic and eosinophilic, infiltration in all layers but particularly of submucosa. *Large intestine:* (G) cecum and first portion of ascending colon: marked edema; subserosal hemorrhages; (M) massive leukocytic and histiocytic infiltration, particularly in submucosa and subserosa.

Case 13

The patient was a white male, 26 years of age. Duration of hepatitis, 49 days. (See page 482 for clinical course.)

Pathologic Findings. Esophagus: (G) congestion of lower third; (M) slight erosion; submucosal scattering of lymphocytes. *Stomach:* (G and M) normal. *Duodenum and papilla of Vater:* (M) moderate submucosal edema; moderate degree of leukocytic and mononuclear cell infiltration in submucosa. *Ileum and jejunum:* (G) normal. *Cecum:* (G) large hemorrhagic areas in submucosa; edema; (M) phlegmonous infiltration.

Case 22

The patient was a white male, 28 years old. Duration of hepatitis, 17 days.

Pathologic Findings. Esophagus and stomach: (G) normal. *Small intestine:* (M) moderate degree of submucosal edema; sparse scattering of histiocytes and lymphocytes. *Large intestine:* (G) cecum was markedly edematous; ascending colon, appendix and terminal ileum not noticeably involved; (M) surface of cecum intact; mucosal glands well preserved; submucosa greatly distended; its thickness exceeded 1 cm.; fibers of muscle layer forced apart; subserosal tissue loosely textured. Throughout submucosa and subserosa, diffuse infiltration with histiocytes and leukocytes. Lower colon had edematous walls, but no cellular exudate had occurred.

Case 29

This patient was a white male, 35 years of age. Duration of hepatitis, 45 days.

Pathologic Findings. Esophagus: (G) superficial erosion; congestion; (M) submucosal edema; foci of lymphocytes and plasma cells. *Stomach:* (G and M) normal. *Duodenum and papilla of Vater:* (G) moderately edematous; slight submucosal edema; sparse scattering of leukocytes. *Ileum and jejunum:* (G) no significant changes. *Large intestine:* (G) almost all portions, but particularly cecum, showed massive edema but no ulceration; (M) mucosa intact; all layers edematous; diffusely infiltrated with leukocytes and histiocytes.

Case 43

The patient was a white male, 24 years old. Duration of hepatitis, 20 days.

Pathologic Findings. Stomach: (G) normal. *Duodenum and papilla of Vater:* (G) no significant changes. *Ileum and jejunum:* (G) no significant changes. *Large*

intestine: (G) mucosa intact; marked congestion and edema; (M) extreme edema and diffuse cellular infiltration throughout all layers; thickness of wall ranged from 8 to 10 mm. Mucosa unbroken; glands normal; supporting stroma infiltrated with scanty numbers of plasma cells, histiocytes and granulocytes. Submucosa the main site of inflammatory edema; its fibers were widely separated and thickly invaded with leukocytes and histiocytes. Venules had collars of exudative cells. In muscular and subserous layers, phlegmonous changes were of somewhat less degree than in submucosa. Muscle fibers in many patches showed degenerative changes.

Case 54

The patient was a white male, 24 years of age. Duration of hepatitis, 36 days.

Pathologic Findings. Stomach and small intestine: (G) hyperemia; (M) moderate degree of edema of small intestine. *Large intestine:* (M) moderate degree of edema and phlegmonous inflammation, most marked in submucosa and subserosa.

Case 55

This patient was a white male, 23 years old. Duration of hepatitis, 29 days.

Pathologic Findings. Esophagus: (M) two superficial ulcers at cardiac end; subjacent tissue edematous and massively infiltrated with lymphocytes and leukocytes; base of ulcer covered with fibrin. *Stomach:* (G) multiple petechiae. *Small intestine:* (G) multiple petechiae. *Large intestine:* (G) marked edema, particularly of cecum and ascending colon; multiple petechiae; (M) mucosa preserved; extensive phlegmonous infiltration, particularly of cecum.

Case 59

The patient was a white male, 26 years old. Duration of hepatitis, 38 days. (See page 493 for clinical course.)

Pathologic Findings. Esophagus: (G) no significant changes. *Stomach:* (M) edema of submucosa; scattered inflammatory cells. *Duodenum:* (G) slight edema. *Ileum:* (G) lower portion markedly edematous but not congested. *Large intestine:* (G) cecum, ascending and transverse colon; intense edema; no congestion; (M) diffuse phlegmonous infiltration of cecum and ascending colon; remainder of colon edematous.

Case 61

The patient was a white male, 25 years of age. Duration of hepatitis, 40 days.

Pathologic Findings. Esophagus: (G) several small longitudinal erosions in lower third. *Stomach:* (G) multiple superficial ulcers. *Duodenum and papilla of Vater:* (G) edematous; congested. *Ileum and jejunum:* (G) edematous. *Large intestine:* (G) marked edema, especially in sigmoid; here wall averaged 1 cm. in thickness, section had a yellowish gray appearance; mucosa preserved; (M) entire wall densely and diffusely infiltrated with granulocytes, including many eosinophils, and macrophages; exudate most conspicuous in submucosa but all layers crowded with cells; in appendix, same changes but less marked.

Case 94

This patient was a white male, 35 years of age. Duration of hepatitis, 50 days.

Pathologic Findings. Esophagus and stomach: (G and M) no significant changes. *Duodenum and papilla of Vater:* (G) wall very edematous; mucosa hyperemic. *Jejunum:* (G) slightly edematous; mucosa congested. *Large intestine:* (G) walls very soggy; mucosa not ulcerated; (M) mucosa intact; submucosa extremely edematous with numerous leukocytes and histiocytes; remainder of wall moderately edematous and diffusely infiltrated with inflammatory cells. *Ileum:* (G)

moderately edematous; (M) edematous; wall diffusely infiltrated with leukocytes and histiocytes.

Case 97

The patient was a white male, 50 years old. Duration of hepatitis, 74 days.

Pathologic Findings. Esophagus: (G) no significant changes. *Stomach:* (G) moderately edematous; (M) edema; scattered histiocytes in submucosa and muscularis. *Small intestine:* (G) walls thickened and moist; (M) moderate edema of submucosa and serosa; scattered histiocytes in subserous layer. *Large intestine:* (G) wall thickened and markedly edematous; mucosa thrown up in folds, giving a coarse nodular appearance; (M) marked edema of entire wall with diffuse infiltration of granulocytes, histiocytes, lymphocytes and plasma cells; process was most marked in submucosa and muscularis.

Case 100

This patient was a white male, 27 years of age. Duration of hepatitis, 93 days. (See page 485 for clinical course.)

Pathologic Findings. Esophagus, stomach and small intestine: (G) no significant changes. *Large intestine:* (G) appendix was normal; wall of ascending colon markedly edematous; no ulceration; (M) conspicuous edema of submucosa and infiltration with histiocytes, granulocytes and plasma cells.

Case 103

This patient was a white male, 22 years old. Duration of hepatitis, 49 days.

Pathologic Findings. Esophagus: (G) no gross ulceration; surface discolored by reddish black material; (M) superficial ulceration with conspicuous polymorphonuclear and histiocytic reaction; underlying tissue slightly edematous and sparsely infiltrated with inflammatory cells. *Stomach:* (G) multiple petechiae; (M) no significant changes. *Duodenum and papilla of Vater:* (G) congestion of mucosa. *Jejunum and ileum:* (G) intense congestion of mucosa; (M) congestion. *Large intestine:* (G) normal; (M) edema of submucosa; diffuse infiltration with leukocytes and histiocytes.

Case 107

The patient was a white male, 26 years of age. Duration of hepatitis, 50 days.

Pathologic Findings. Stomach: (G) no significant changes. *Duodenum:* (M) diffuse sprinkling of lymphocytes, histiocytes and occasional eosinophile cells throughout all layers. *Ileum and jejunum:* (G) no noteworthy changes. *Large intestine:* (G) normal; (M) edema of submucosa, diffuse infiltration with granulocytes and histiocytes.

Case 112

This patient was a white male, 38 years old. Duration of hepatitis, 40 days.

Pathologic Findings. Esophagus, stomach and small intestine: (G) no significant changes. *Large intestine:* (G) subserosal hemorrhages in descending colon and sigmoid; (M) marked edema; diffuse inflammatory reaction throughout wall; mucosa intact.

Noninflammatory Edema of the Intestine

In addition to the 17 cases with phlegmon, there was noninflammatory edema of small and large intestines in 26 other cases. The edema varied in degree, and involved especially the submucosa (Fig. 53). In all of the cases there was associated ascites.

Ulceration of the Esophagus

The lower one-third of the esophagus in many cases showed changes such as are frequently regarded as due to post-mortem autolysis. The mucosal surface had partly sloughed away, and there were small longitudinal erosions, often covered by chocolate brown débris. Because these lesions are so commonly looked upon as post-mortem changes, material for histologic examinations was available in only 20 cases. In 9 of them, there was superficial ulceration, accompanied by an inflammatory reaction that usually extended to the muscularis, and more rarely throughout the entire wall. The appearance in a representative case is shown in Figure 54. Here, small superficial erosions were present at the cardio-esophageal junction. The denuded tissue was the site of an acute inflammatory reaction that extended laterally beyond the eroded portions and down to the muscularis.

Erosion of this type is too well known to merit further description. It probably occurred more often in the present series than the figures indicate. Most writers on the subject agree that the action of the gastric juice plays an important part in the pathogenesis of esophageal ulceration.[55-57] It is when vomiting is protracted that erosions occur. Even nausea, without vomiting, by relaxation of the cardiac sphincter and regurgitation of gastric juice may induce the lesion. Nausea and vomiting alone, however, are not sufficient; poor circulation in the esophagus, and a state of debilitation of the patient appear to be prerequisites. These factors—nausea, protracted vomiting, debilitation and, probably, impaired circulation in the esophagus (due to interference with the circulation through the liver)—are found in nearly all fatal cases of epidemic hepatitis.

Hemorrhagic Phenomena

As in other forms of hepatic disease, hemorrhages were found in epidemic hepatitis. The location of the hemorrhages in 109 cases is shown in Table XIX. Microscopic hemorrhages are not included in this tabulation. Most common were hemorrhages in the intestinal tract,

TABLE XIX

Location of Hemorrhages in 109 Cases of Epidemic Hepatitis

Location	No. of cases	Per cent of cases
Intestines or mesentery	80	73
Lungs	74	68
Heart	43	39
Kidney	14	13
Skin	8	7
Brain	5	5

which occurred in approximately three-fourths of the series. In degree, they varied from petechiae beneath the serosa and in the mucosa to larger hemorrhages, which were usually at the attachment of the gut to the mesentery or mesocolon (Fig. 63).

Almost as frequent were hemorrhages in the lungs, in which dark red, almost black, hemorrhagic patches with ill-defined outlines were scattered through all lobes. The bronchial walls were dusky red. Sometimes the supporting tissue around the larger vessels was hemorrhagic. Microscopically, in large areas the air sacs and bronchial walls were flooded with blood. It is probable that the pulmonary hemorrhages predispose to bacterial invasion, for in a great majority of cases foci of terminal pneumonia were found at autopsy.

Next in frequency were hemorrhages in the heart, where they were located in the epicardium and, commonly, beneath the septal endocardium of the left ventricle, near the undefended space (Fig. 62).

In the kidney, the hemorrhages were usually pelvic in location. In other organs hemorrhages were found more rarely.

Bone Marrow

Bone marrow specimens were available for histologic examination in 22 cases, the tissue having been selected usually from rib, sternum, or vertebra. This relatively small series fortunately represents early cases, cases of average duration and others with a protracted course. When one takes into consideration the variables dependent on the age of the patient and the site of the particular specimen, certain generalities can be established.

In most instances there was a mild to moderate degree of hyperplasia (Fig. 64) due more to increased activity of the erythrocytic than of the granulocytic series. Megakaryocytes were normal in structure and distribution and varied in number with the degree of general hyperplasia. There was no disturbance of maturation in the several developmental series, and most cells were found in the midstage (*i.e.*, erythroblasts, myelocytes) or beyond. Degenerative changes were not evident. Pigment, presumably hemosiderin, was often noted within histiocytes.

Certain cases displayed maximal hyperplasia, fat being completely replaced by hematopoietic tissue, which in some instances was predominantly erythroid (Fig. 66), in others myeloid (Fig. 65). The changes bore no constant relation to length of disease. They suggest, however, that a certain degree of hyperplasia of bone marrow occurs at all stages of fatal epidemic hepatitis, probably as the combined result of hemorrhages and of hepatic destruction.

Kidneys

In the majority of cases the kidneys were swollen, flaccid, and bile-stained. The combined weights, tabulated in 88 cases, are as follows: Below 300 gm., 1 case; 300 to 399, 25 cases; 400 to 499, 36 cases; 500 to 599, 17 cases; over 600 gm., 9 cases. The kidneys were therefore considerably swollen, *i.e.*, they weighed over 400 gm. in 70 per cent of the cases. Microscopically, the lesions in most of the kidneys were typical of cholemic nephrosis. The glomeruli were well filled and normally cellular. Precipitated protein in the capsular space, frequently present, indicated alterations in permeability of the filtering capillaries. Pigmented casts lay in the lumina of various parts of the tubular system, particularly in the distal convoluted and the collecting tubules (Fig. 59). Degenerative changes of varying degree were evident, ranging from simple "cloudy swelling" to actual necrosis similar to that of mercurial poisoning (Fig. 60). In severely damaged kidneys, regeneration seemed to go hand in hand with destruction, and it was common to see tubules relined by flat epithelium (Fig. 61).

Cholemic nephrosis has received much less attention than the condition merits.[58-60]

Testis

Cirrhosis of the liver is not infrequently associated with atrophy of the testis. It is therefore a matter of much interest to examine this organ in cases of fatal epidemic hepatitis. Material was available in 38 cases. In 7 cases the organs were found to be completely atrophic and fibrotic: in 5 some degree of disturbance of spermatogenesis was evident, and in 26 cases the testes were normal. In the 7 cases of complete atrophy and fibrosis, the duration of hepatitis was from 26 to 64 days. The testicular process in all appeared to be of much longer duration.

In the 5 cases in which there was a varying degree of interference with spermatogenesis, there was some edema of stroma but without fibrosis. Hepatitis had been present from 52 to 76 days in 4, and 138 days in one. In these cases it is not certain whether the testicular changes can be related to the hepatic damage.

It may here be pertinent to recall the relation of the liver to the testis. The liver is important in the metabolic disposal of sex hormones. When, because of hepatic insufficiency, this disposal does not take place, estrogens accumulate; these substances, in excessive concentration, very probably damage the sperm-forming cells of the testis and thus may lead to severe atrophy.[61]

In a recent study of 34 cases of hepatic failure other than cirrhosis (carcinoma, amyloidosis, hemochromatosis) Morrione [62] found that in

order to result in atrophy of the testis, damage in the liver must be severe, extensive and long-standing. Whether in any case of the present series the testicular damage can be related to the hepatic damage is doubtful. Study of much additional material is required to aid in the solution of this complicated problem.

Brain

Grossly, the brain showed little alteration, except edema. In 62 cases, blocks from representative areas were available for microscopic examination. The changes found were of two kinds. One was acute degeneration of ganglion cells, the other inflammatory reaction around vessels and in the meninges.

The changes in the ganglion cells were of types such as occur commonly in many different diseases (Figs. 67 to 69). Satellitosis around damaged cells was rare. The ganglion cell changes were irregular in distribution; no part of the brain was especially affected. Occasionally, isolated cells were completely destroyed; their remnants usually were invaded by glial elements (Figs. 70 and 71). In a few instances there were found tiny glial nodules (Fig. 72). Otherwise, glial reactions of any kind were sparse.

The vessels had normal walls; hemorrhages were rare. Edema of varied degree was usually present. There were nowhere any areas of demyelination. All these changes are nonspecific.

A different and more important lesion was found in the basal meninges and around the vessels of the brain stem (including the hypothalamus), in the periventricular system and the nucleus basalis. In these regions occasional small vessels were cuffed by lymphocytes (Fig. 73). Such perivascular infiltration was never pronounced. Figure 74 is representative of the average degree. The meninges showed a similar and often more diffuse lymphocytic infiltration (Figs. 75 and 76). The vessel walls were never necrotic. The lesions did not occur in the cortex or the subjacent white matter.

The perivascular and meningeal infiltrations were present in approximately 15 per cent of the brains examined. The lesions are sufficient in degree to be considered a mild meningo-encephalitis.

Pathologic Physiology of Fatal Epidemic Hepatitis

It is beyond the scope of this paper to discuss all of the varying functional faults encountered in fatal cases of epidemic hepatitis. Rather, I shall briefly discuss the mechanism underlying the jaundice, the ascites, the hemorrhagic phenomena and the cerebral manifestations.

Jaundice

Jaundice indicates a disturbance of the balance between the rates of bile pigment production and its excretion by liver cells into the biliary system. Regarding the pathogenesis of jaundice there are two main schools. By one, following the teaching of McNee,[63] three types of jaundice are recognized: hemolytic jaundice, toxic jaundice and obstructive jaundice. In hemolytic jaundice the rate of bile pigment formation, due to excessive destruction of red corpuscles, is so great that it exceeds the rate with which the liver cells can modify and then eliminate the pigment. Hence, an excess of bilirubin accumulates in the circulation and tints certain tissues. In the second type of jaundice, the toxic variety, the liver cells are severely damaged or actually killed and hence cannot take up the bilirubin of normal hemoglobin breakdown from the circulating blood. In the third type, obstructive jaundice, the rate of pigment formation by the reticulo-endothelial cells is normal and the liver cells are able to secrete the pigment into the intracolumnar canaliculi. Due to obstruction somewhere in the excretory duct system, the delicate canaliculi become distended beyond their capacity and rupture, spilling bile into the space of Disse or directly into the circulating blood.

A somewhat different view concerning the mechanism of jaundice is expressed by Rich.[64] He recognizes only two main types: retention jaundice and regurgitation jaundice. The former is practically synonymous with hemolytic jaundice of other writers. It occurs in various anemias and in any condition associated with increased destruction of red blood cells. The other form, regurgitation jaundice, is due to a reflux of bile from the intralobular canaliculi into the blood stream. It results from rupture of the delicate canaliculi, brought about either by necrosis of liver cells or by obstruction of the outflow of bile anywhere within the biliary system. The regurgitation jaundice of Rich, therefore, includes both the toxic and obstructive variety. He believes catarrhal jaundice to be a form of regurgitation jaundice of unknown pathogenesis.

McNee, Rich, and other students of jaundice agree that the causes of jaundice cannot always be placed in these separate compartments; there is often a combination of several factors.

Catarrhal jaundice for many years was thought to be due to obstruction at the lower end of the biliary duct system. In contrast, the jaundice of idiopathic yellow atrophy of the liver—a condition formerly regarded as an unrelated disease—was usually attributed to damage or destruction of liver cells. It has been shown, however, that catarrhal jaundice and yellow atrophy of the liver are not different diseases but

different forms of the same disease, namely, epidemic hepatitis. If this be true, then it may be expected that in both forms the pathogenesis of jaundice is the same. Actually, in both forms, the cause of jaundice appears to lie principally in obstruction of the intralobular canaliculi by bile thrombi. In biopsies reported by Scandinavian and British investigators, and in rare post-mortem examinations of patients who have died from accidental causes, the liver in catarrhal jaundice has always shown, within bile canaliculi, plugs of altered and inspissated bile. In fatal cases of epidemic hepatitis, *i.e.*, in "idiopathic" yellow atrophy, these plugs occur in great numbers within the intralobular canaliculi. In contrast the extralobular ducts, small and large, are patent. No doubt, injury to the liver may be an additional factor in early stages of epidemic hepatitis in both its benign and malignant forms. But it is more than probable that mechanical obstruction of the intralobular canaliculi is the most important cause of the persistent jaundice of epidemic hepatitis.

Ascites

Ascites is a common event in fatal epidemic hepatitis. It occurs in approximately two-thirds of the cases. Most often ascites appears rather suddenly, during the final phase of the disease. It should be emphasized, however, that it may occur also, though less often, in patients who recover.

The mechanism of ascites is complex. At least two factors are usually involved: (1) alteration of the plasma proteins which leads to lowering of the colloid osmotic pressure, and (2) increase in portal blood pressure due to interference with venous flow through the liver. The resulting states will, in turn, bring about anoxic increase in capillary permeability.

It is well known that extensive damage to the liver tends to lower the quantity of the plasma proteins and alter their relative proportion.[65] In many cases of this series, the plasma proteins were depleted. Ascites, however, often appeared before the protein level dropped. This was especially true when the course of hepatitis was rapid. In a considerable number of cases the plasma proteins showed little alteration. Moreover, the accumulation of fluid was commonly limited to the abdominal cavity; general edema was rare. There is, however, no constant relation between the level of plasma proteins and the colloid osmotic pressure of the blood. This subject was discussed in a recent paper by Butt, Snell and Keys.[66] These investigators brought together experimental and clinical evidence which indicates that in hepatic disease the proteins may be altered both in quantity and in quality.

They were unable to demonstrate a constant "edema level" by measuring the colloid osmotic pressure. They cited the results of unpublished experiments of Bollman, who found no direct relation between the appearance of ascites and any exact serum protein concentration or level of the colloid osmotic pressure. But Bollman did find the rapidity of appearance of ascites more directly related to the extent of the hepatic injury. All these facts suggest that fall in the plasma proteins or in osmotic pressure is usually not the main cause of ascites in fatal epidemic hepatitis.

We may now consider another factor that may be responsible for ascites, namely, obstruction to the flow of blood in the liver. In epidemic hepatitis many efferent veins are severely injured. There is marked endophlebitis, sometimes thrombosis and complete obliteration. Moreover, there are changes in the distribution of blood within the liver. Where new parenchyma has been formed the tissue is markedly ischemic. Where tissue has been destroyed there is great engorgement, perhaps stagnation. There is, therefore, the possibility that even in early stages of hepatitis portal flow may be impaired.

That portal obstruction actually exists is shown by comparison of spleens from patients with ascites and from those without ascites. The spleen in the ascitic group is enlarged twice as often as in the group without ascites. This enlargement may be regarded as evidence of portal obstruction. Although this does not furnish positive proof, it suggests that obstruction to portal flow is at least one factor in the pathogenesis of ascites.

Besides mechanical obstruction, other factors may be concerned. The liver normally stores a large proportion of ingested water.[67] Destruction of much of the parenchyma in fatal hepatitis may interfere with the function of water storage and thus contribute to the establishment of ascites.

Hemorrhagic Phenomena

The hemorrhagic phenomena of epidemic hepatitis are doubtless due to disturbance in prothrombin formation in the damaged liver. The relation of hemorrhage to liver damage has been reviewed in recent papers.[68, 69]

Cerebral Manifestation

Mental and nervous manifestations are almost invariably present during the final phase of fatal hepatitis. These are somewhat paralleled by the symptoms observed in experimental animals subjected to complete removal of the liver. In such animals the symptoms produced, and the death of the animals, must be attributed chiefly to hypoglycemia which takes places after the liver, the chief storehouse of

glycogen, is removed. However, animals in which some hepatic tissue remains do not show the typical symptoms that are present when the liver is removed completely. Death in such animals cannot always be attributed to hypoglycemia.[65]

Severe hepatic injury must inevitably lead to serious disturbances in the chemical composition of the body fluids, on the stability of which depends the normal state of all tissue. It seems reasonable to assume that it is the general imbalance of tissue fluids rather than the disturbance of a single component, such as sugar, which is responsible for the cerebral manifestations and for death.

The anatomic background of the cerebral symptoms is ill-defined. In this series, two main kinds of changes were found on microscopic examination. One was acute nonspecific degeneration of ganglion cells, such as accompanies many different diseases. These changes occurred in all cases. The other was a mild form of meningo-encephalitis located particularly in the brain stem, including the hypothalamus, the periventricular system and the nucleus basalis; the cortex and the subjacent white matter remained untouched. These changes occurred in approximately 15 per cent of the brains.

The lesions are different from those found in virus diseases, such as measles, mumps, or vaccinia. In these, foci of demyelination and often necrosis of small arteries are characteristic; such alterations are not found in cases of hepatitis.

The cerebral symptoms of hepatitis have some points in common with Wernicke's syndrome, in which the clinical manifestations are lethargy or drowsiness terminating in coma, and sometimes periods of excitement. Frequently, these nervous manifestations are associated with vomiting.[71] In the pathogenesis of Wernicke's syndrome, vitamin deficiency, particularly deficiency in Vitamin B_1, is thought to play an important part. Deficiencies of several vitamins probably occur when the liver is as extensively damaged as it is in fatal hepatitis. However, anatomically the lesions of Wernicke's encephalopathy differ from those found in cases of epidemic hepatitis. In Wernicke's encephalopathy the lesions consist mainly of vascular proliferations, a varying degree of glial proliferation, and absence of perivascular lymphocytic cuffing.[70, 71] In hepatitis, on the other hand, vascular and glial proliferations are inconspicuous or absent, but perivascular lymphatic infiltration is present. Because of these differences there is no ground for believing that the cerebral lesions of hepatitis are the result of vitamin B_1 deficiency.

We may now consider whether the changes in the brain may result from toxic substances passing through the damaged liver. The portal

blood always contains an abundance of toxic products of digestion which in the normal liver are detoxified. Through the greatly damaged liver of fatal hepatitis they may pass unaltered. Certain experiments of Baló and Korpássy [72] have a bearing on this point. When dogs with Eck's fistula were fed meat, 6 of 8 animals presented symptoms of encephalitis. In the brains of 3 dogs, histologic changes were found which corresponded to nonsuppurative encephalitis; they were located particularly in the striate body. These lesions were of similar kind, but of greater degree, than those found in hepatitis. Baló and Korpássy concluded that the encephalitis in their experimental animals was due to intoxication caused by exclusion of the liver.

It seems probable that in fatal hepatitis the detoxifying function of the liver is so altered as to permit the circulation of substances normally bound or destroyed in this organ. The working hypothesis is proposed that the cerebral changes of hepatitis result chiefly from loss of the detoxifying function of the liver.

Summary

This study is based upon 125 fatal cases of hepatitis which occurred in the U. S. Army during the epidemic of 1942.

In an historical summary it has been shown that hepatitis is not a new disease; many epidemics have occurred. In all epidemics the clinical picture has been remarkably similar. The mortality has always been low, from 0.2 to 0.4 per cent.

Prior to World War I there was little information concerning the lesions of epidemic hepatitis. It has gradually come to be recognized that the lesions in the livers of fatal cases correspond to those long known as idiopathic acute yellow atrophy. Nevertheless, the view that yellow atrophy and epidemic hepatitis are but two extremes of the same disease has not been accepted by all. The present studies leave little doubt that idiopathic yellow atrophy represents the end stage of fatal epidemic hepatitis.

The clinical course in the fatal cases has been analyzed. There were three distinct phases: pre-icteric or initial, intermediate, and final. In the great majority, the initial phase lasted 7 days or less, the intermediate phase no more than 26 days, the final phase no more than 10. The initial symptoms were the same as those in cases of the nonfatal disease. The intermediate phase began with the onset of jaundice; during its early stages there was no indication that the disease was not going to run its usual benign course. However, in the majority there was a sudden dramatic change which initiated the final phase of the disease; it usually was marked by the development of cerebral manifestations, persistent vomiting and ascites. The cerebral symptoms

were, principally, lethargy and coma alternating with excitement and delirium.

The patient was usually afebrile, except during the terminal stage. The pulse was rarely slow. The blood pressure was normal, except during periods of excitement in the final phase.

During the stages preceding the final phase the liver was usually enlarged and tender. Later it shrank rapidly.

As the disease progressed, moderate anemia appeared. In all but the final stage, there was usually slight leukopenia with relative lymphocytosis.

The chief lesions were found in the liver. They were those typical of yellow atrophy. Characteristically, involvement of the liver was not uniform. In large areas all the parenchyma was destroyed, leaving only skeletal remnants of the lobules, whereas elsewhere destruction was incomplete. The remains of lobules were outlined by small proliferating bile ducts. The destruction affected only the liver cells, the framework and sinusoids remaining unaltered. Scarring did not occur. It is characteristic of the destructive process that the dead cells were removed rapidly. In the areas of destruction there was an inflammatory reaction that persisted for a long time. Macrophages filled with lipofuscin were particularly prominent. The efferent veins were the site of marked endophlebitis.

Hyperplasia of surviving cells led to the formation of much new tissue which grossly appeared coarsely nodular or tumor-like. Microscopically, this tissue almost never had a normal lobular structure. The new parenchyma was formed almost exclusively from pre-existing liver cells. However, there is some morphologic evidence that liver cells are sometimes derived from bile ducts. While it is impossible to decide on morphologic grounds whether bile ducts can actually form liver cells, it is probable that they do so.

The newly formed parenchyma was markedly ischemic and overladen with bile, due to obstruction in the intralobular canaliculi. The extralobular bile ducts were normal. There was no evidence of progressive destruction of tissue. But in some areas the newly formed parenchyma degenerated because of ischemia, bile stasis, and, perhaps, accumulation of metabolic waste products.

The gallbladder showed no significant change.

The regional lymph nodes of the liver were edematous and often hyperplastic.

The spleen in the majority of instances was enlarged. In early stages, enlargement was due to cellular proliferation, and in later stages to congestion.

The bone marrow usually was moderately hyperplastic.

In the gastrointestinal tract edema was commonly found, and in about 15 per cent of the cases there was a phlegmonous inflammation, particularly in the cecal region. The phlegmon was probably a late event, as the inflammatory cells were well preserved.

The kidney usually showed the picture of cholemic nephrosis.

In the brain two kinds of changes were present: an acute nonspecific degeneration of ganglion cells, and a mild meningo-encephalitis which occurred in about 15 per cent of the cases.

Hemorrhages were found particularly in the lung, intestine, epicardium, endocardium and kidney. Hemorrhagic phenomena may be attributed to disturbances in vitamin-K metabolism and to changes in the level of prothrombin, both due to destruction of liver.

Ascites occurred in two-thirds of the cases; its frequency was unrelated to the total duration of hepatitis. It usually developed late in the disease. Ascites is thought to be due principally to interference with blood flow through the liver, although other factors may be concerned, such as change in plasma proteins and interference with the water-storage capacity of the liver.

The mechanism of jaundice is believed to be obstruction of the intralobular canaliculi.

The cerebral lesions are believed to be due to loss of the detoxifying function of the liver, although here, too, the exact mechanism is not known.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 88

FIG. 1. Case 24. Duration of hepatitis, 36 days. Under surface of a liver which weighed 1320 gm. The liver is shrunken, particularly the left lobe. The surface of the right lobe shows a number of flat or elevated nodular areas, between which the tissue is finely wrinkled. The surface of the left lobe is deeply furrowed. At the hilum is a cluster of enlarged, edematous lymph nodes. (For microscopic appearance of liver see Fig. 30; hemorrhages in heart and gut of this case are shown in Figs. 62 and 63.)

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FIG. 2. Case 81. Duration of disease, 19 days. Cut surface of a liver which weighed 890 gm. Over one-half of the organ has a fleshy, red appearance: here all liver cells have been destroyed. The yellow nodular patches are relatively ischemic, and are composed of large "lobules" of regenerating tissue. (The microscopic appearance of the red part is shown in Fig. 4.)

FIG. 3. Case 8. Duration of disease, 43 days. Vertical cut section of a liver which weighed 850 gm. The large, red, fleshy area consists entirely of vascular stroma and bile ducts; all liver cells have been destroyed. The remainder of the organ is composed of yellowish green nodular areas of regenerating parenchyma. (The microscopic appearance of the latter is shown in Figs. 27 and 28. For microscopic appearance of the esophagus from this case see Fig. 54.)

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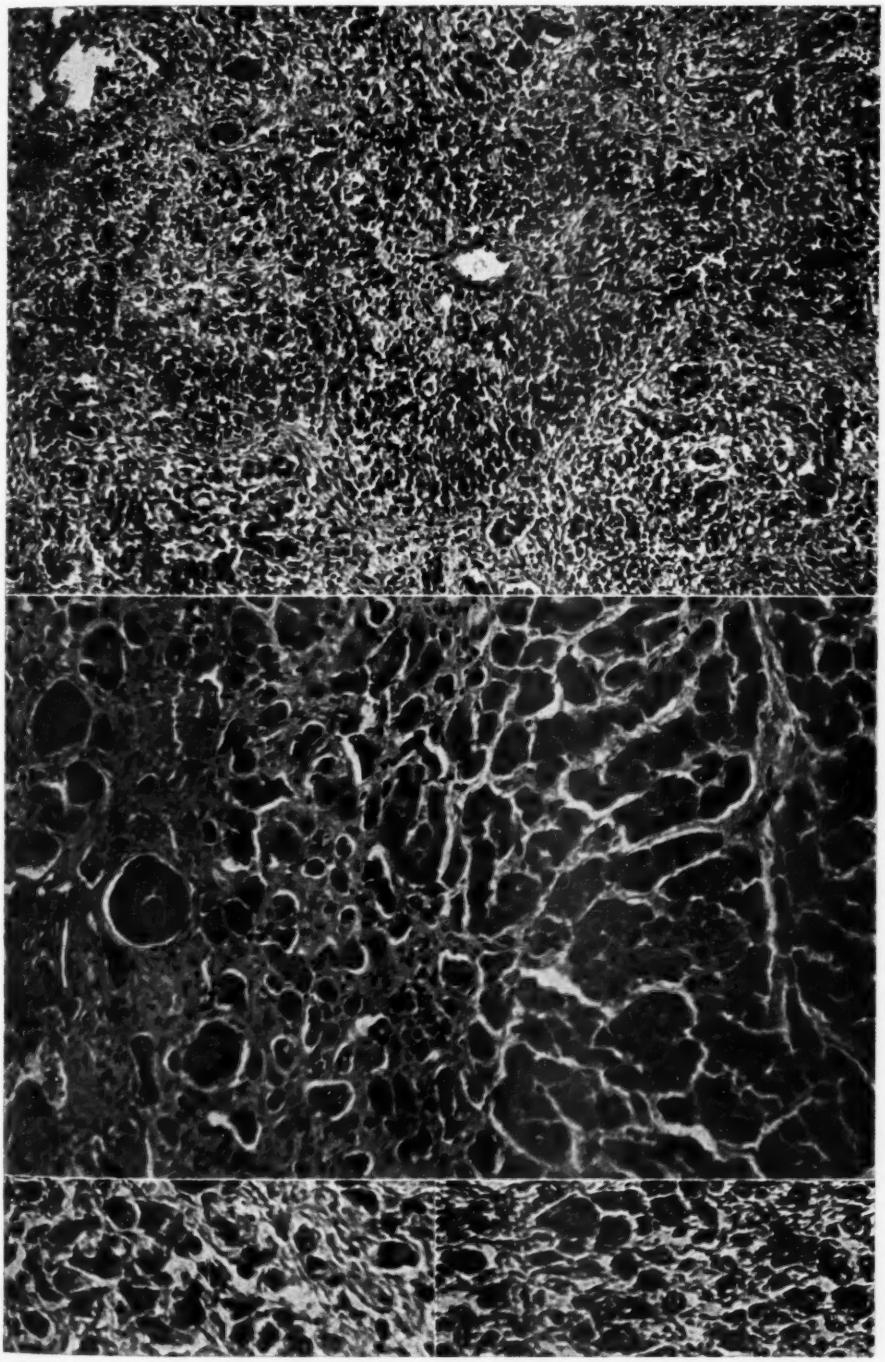
PLATE 90

FIG. 4. Case 81. Duration of disease, 19 days. Microscopic appearance of the red, fleshy area of the liver shown in Fig. 2. The parenchyma has been destroyed. The lobular outlines are indicated by numerous, small, biliary ducts. The sinusoids are greatly engorged. Masson's trichrome stain. × 250.

FIG. 5. Case 84. Duration of disease, 93 days. Microscopic appearance of the pale nodular areas of regenerative hyperplasia shown in Fig. 11. Cords of liver cells form a pseudolobule which is noticeably ischemic. Elsewhere small bile ducts and large tubules composed of hepatic cells are scattered throughout the collapsed stroma; these large tubules are reminiscent of the liver in the early stage of embryonic development. × 250.

FIG. 6a. Case 115. Duration of disease, 18 days. Numerous macrophages with yellow-brown granules are scattered throughout the lobular stroma from which the liver cells have been removed. × 500.

FIG. 6b. A section from an area similar to the one shown in Figure 6a, but stained with Sudan III. The pigment granules take the fat stain well. × 500.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6A
6B

PLATE 91

FIG. 7. Case 104. Duration of disease, 98 days. A branching bile duct, the lumen of which is in continuity with the canaliculi of two atypical and probably regenerated hepatic columns. This appearance suggests a possible transformation of biliary epithelium into liver cells. × 650.

FIG. 8. Case 28. Duration of disease, 21 days. The lumen of a branching bile duct is continuous with that of a tubule composed of liver cells. Surrounded by the epithelium of the bile duct lie several large typical liver cells; as in Figure 7, this suggests a transformation of biliary cells into hepatic cells. The section comes from an area of "red atrophy." × 650.

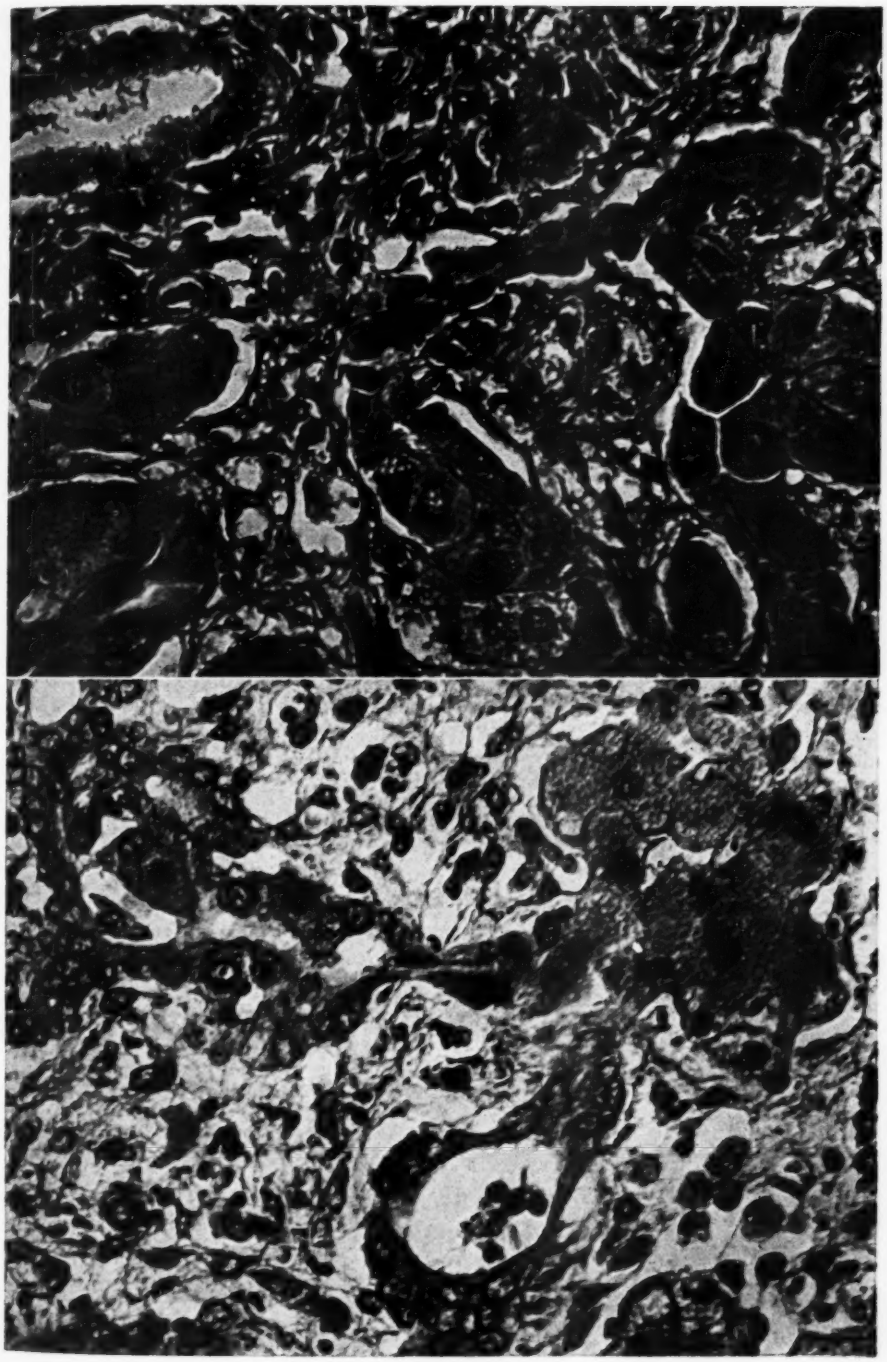

PLATE 92

FIG. 9. Case 92. Duration of hepatitis, 69 days. Weight of liver, 1040 gm. Massive tumor-like areas of hyperplastic regenerated tissue project above sunken patches. The surface of sunken regions has the appearance of coarse-grained leather.

FIG. 10. Case 124. Duration of disease, 37 days. Weight of liver, 800 gm. Under surface of organ shows numerous coarse nodules; the remainder has a smooth or finely wrinkled appearance. (See Figs. 73 and 75 for changes in the brain of this case.)

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PLATE 93

FIG. 11. Case 84. Duration of disease, 93 days. Weight of liver, 1710 gm. The cut surface shows extensive pale areas of regenerated "lobules"; between them lie depressed patches of tissue having a smooth texture and a fleshy appearance. (The microscopic structure of a regenerated "lobule" is shown in Figs. 5, 32, 37 and 46. For structure of the kidney of this case see Figs. 60 and 61; for brain changes see Figs. 71 and 72.)

FIG. 12. Case 76. Duration of disease, 96 days. Weight of liver, 2100 gm. This is an example of massive regenerative hyperplasia limited almost exclusively to the right lobe; the left lobe consists chiefly of collapsed stroma. (See Fig. 20 for appearance of reticulum in left lobe, and Figs. 67, 68, 69 and 76 for changes in the brain.)

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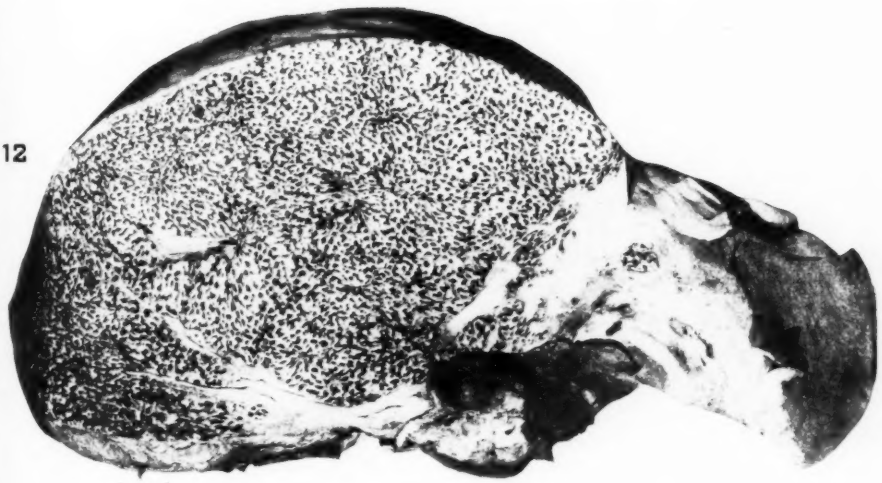

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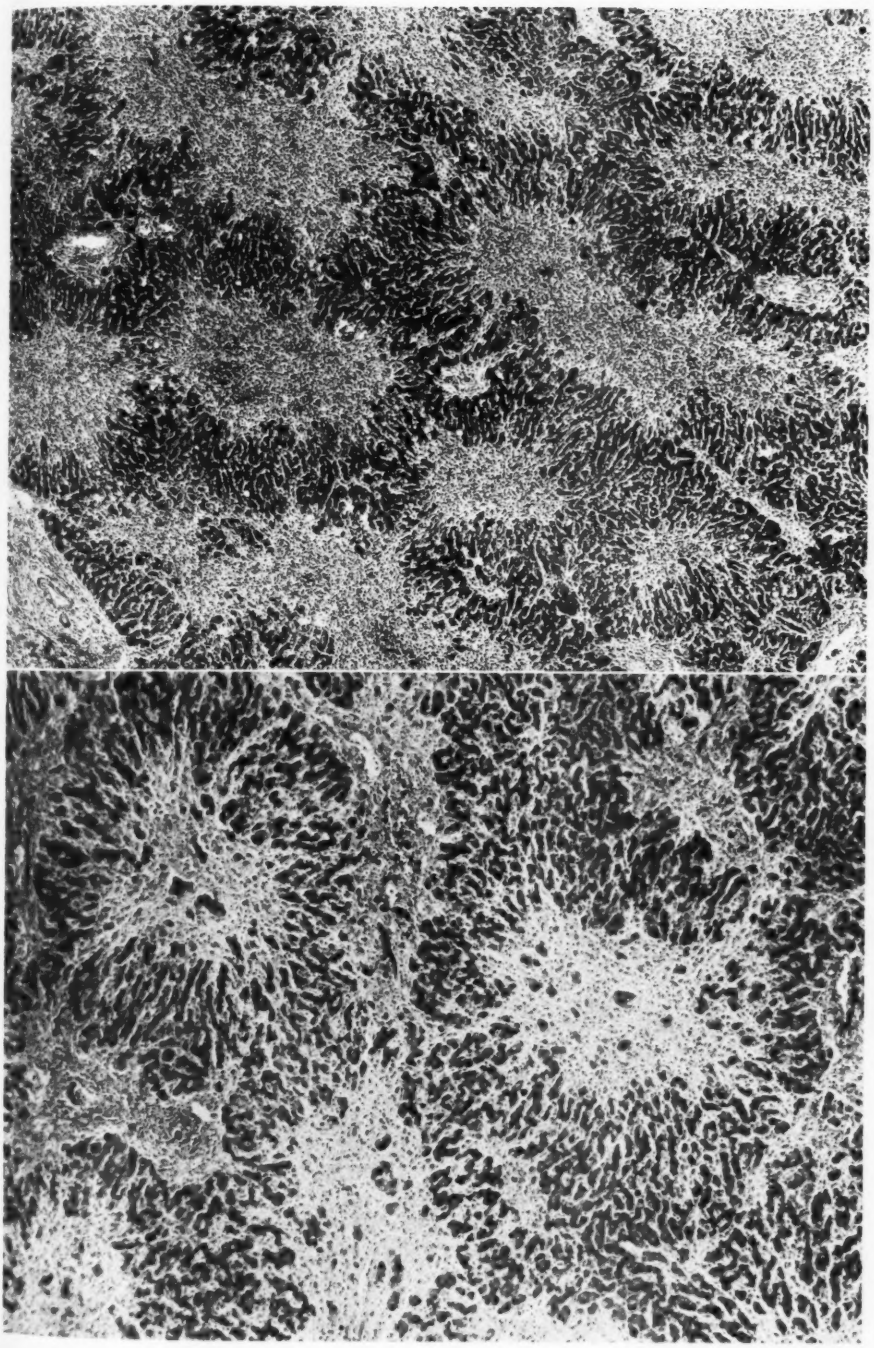
PLATE 94

FIG. 13. Case 4. Duration of disease, 10 days. The central parts of the lobules have been destroyed; the peripheral rim of hepatic cells has remained more or less intact. (See Fig. 16 for details of cellular reaction within the stroma of the central portion of the lobules, Fig. 21 for changes in an efferent vein, Figs. 24, 25 and 26 for early regenerative activity, and Fig. 39 for appearance of portal canals and interlobular bile ducts.) × 35.

FIG. 14. Case 18. Duration of hepatitis, 30 days. The central zones of the lobules are largely destroyed, leaving a peripheral rim of parenchyma. × 120.



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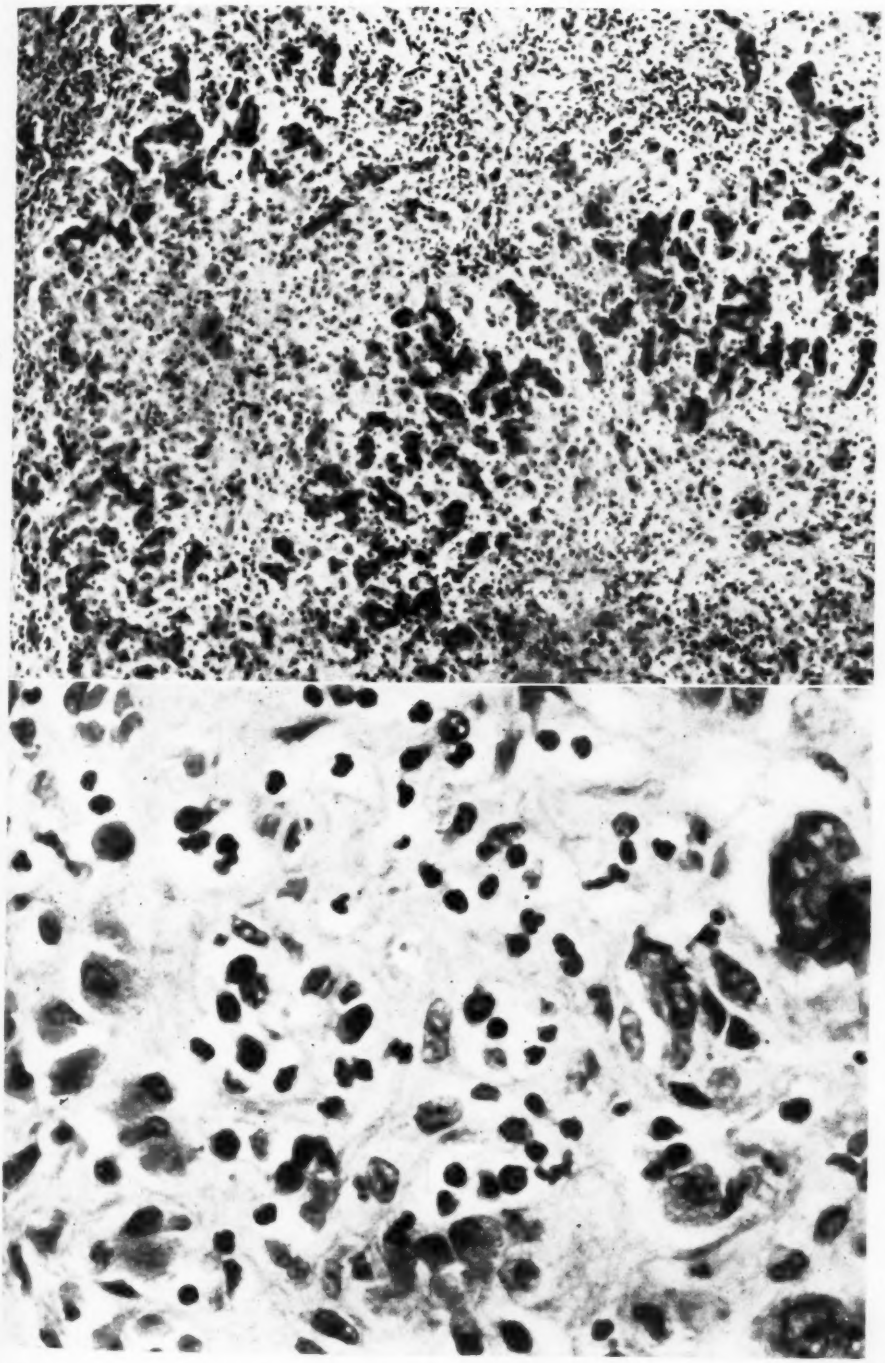
Pathology of Fatal Epidemic Hepatitis

PLATE 95

FIG. 15. Case 15. Duration of hepatitis, 14 days. The figure shows extensive destruction of parenchyma leaving only isolated broken columns of cells at the periphery of some lobules. The stroma is infiltrated with macrophages and exudative cells. (See Fig. 40 for appearance of interlobular and septal bile ducts.) × 135.

FIG. 16. Case 4. Duration of disease, 10 days. The photomicrograph shows the central part of a lobule, the stroma of which is infiltrated with polymorphonuclear leukocytes, lymphocytes, plasma cells and pigmented macrophages. This pigment is lipofuscin. A clump of multinucleated liver cells is evidence of early regenerative activity. (See Fig. 24.) × 810.



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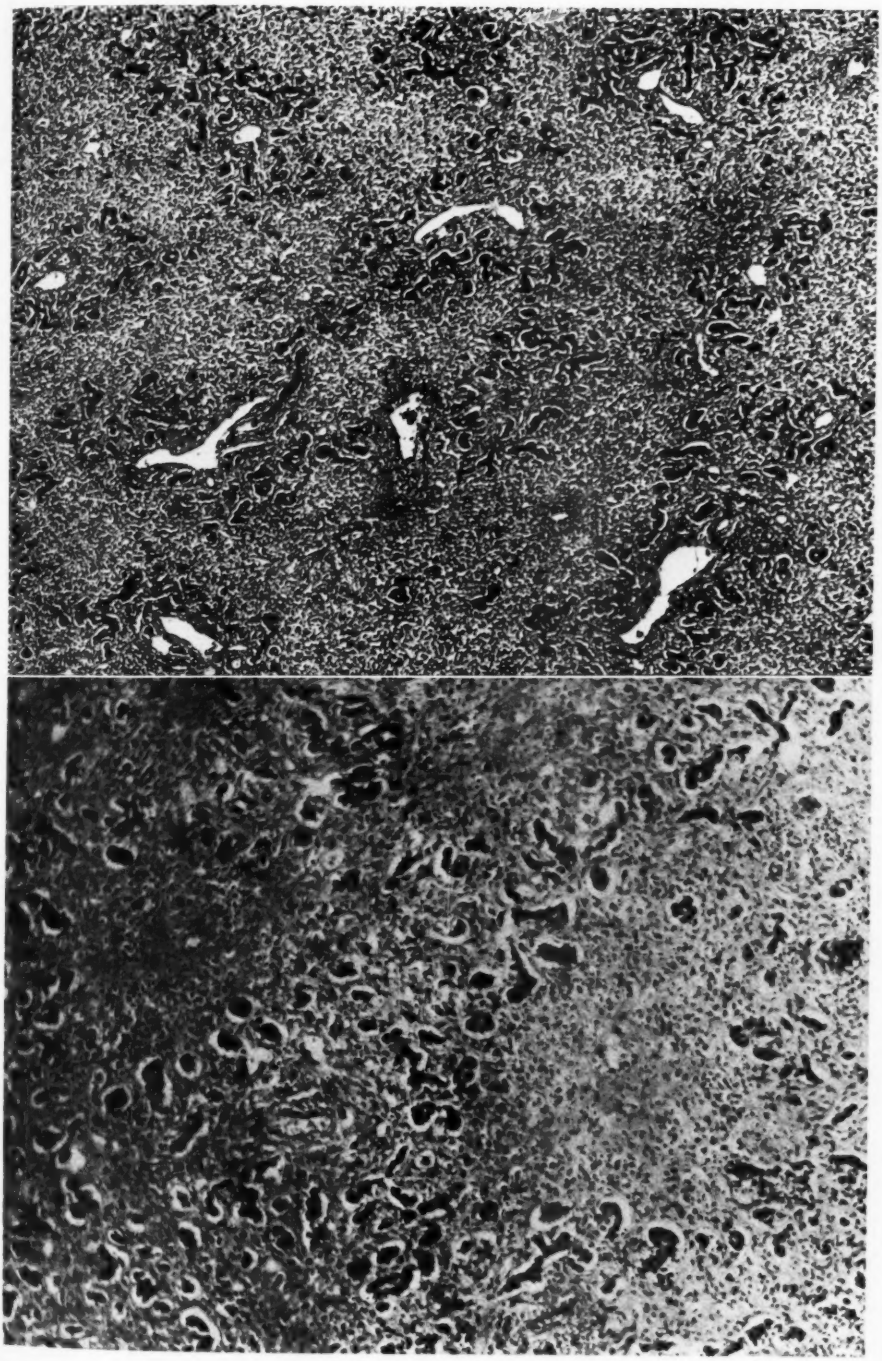
Pathology of Fatal Epidemic Hepatitis

PLATE 96

FIG. 17. Case 79. Duration of hepatitis, 57 days. The section is from an area of "red atrophy." The periphery of the lobular remnants is more or less outlined by stockades of branching bile ducts. (The condition of efferent veins from this case is shown in Figs. 22 and 23.) × 35.

FIG. 18. Case 93. Duration of disease, 76 days. From an area of "red atrophy." The outlines of lobular remnants are indicated by numerous small proliferating biliary ducts. (Elsewhere the liver from this case showed atypical regeneration of parenchyma; Fig. 34.) × 85.



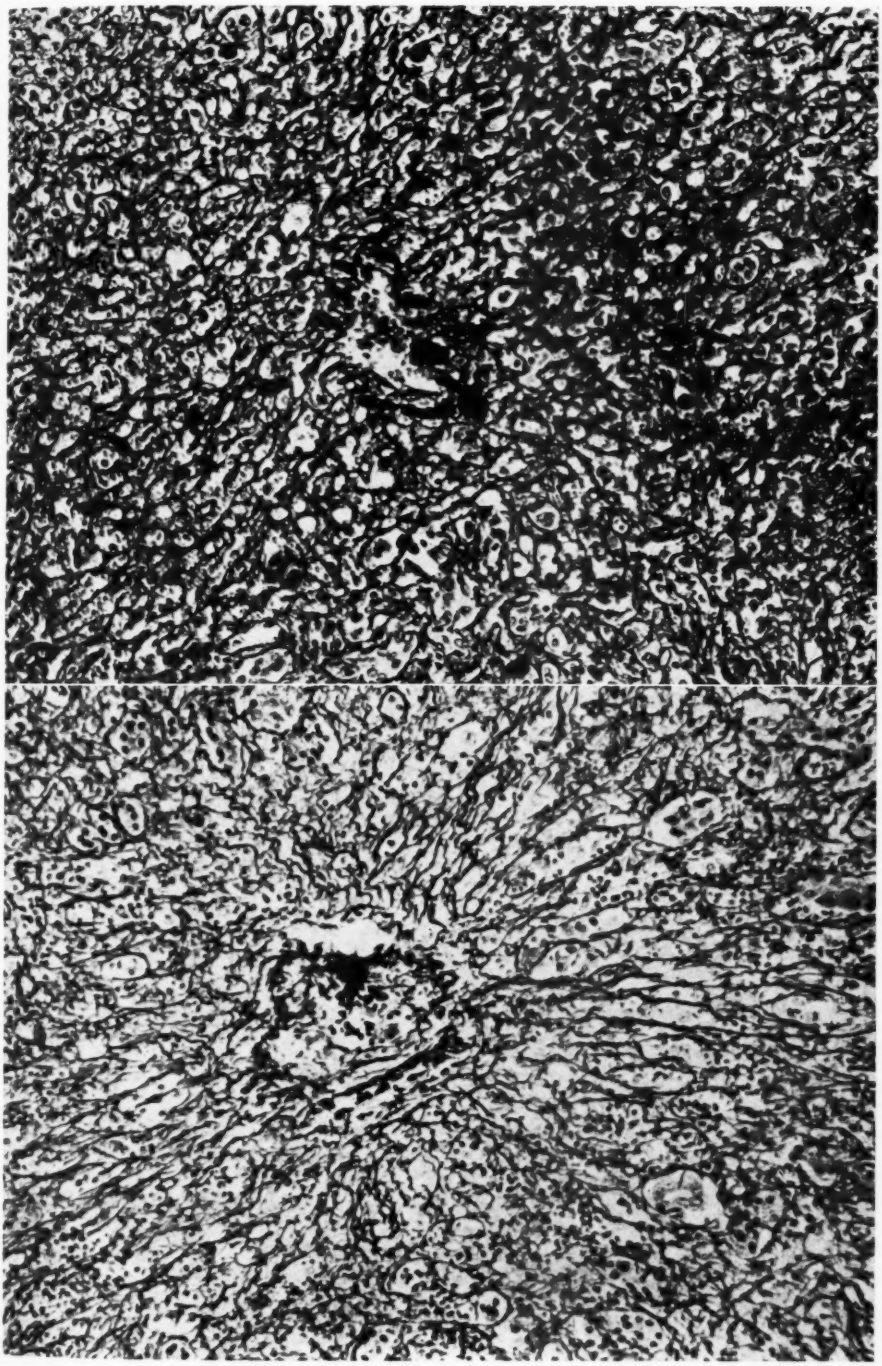
Lucké

Pathology of Fatal Epidemic Hepatitis

Plate 97

Fig. 19. Case 80. Duration of hepatitis, 30 days. The interlobar stroma from an area of "red atrophy" has remained intact. Because of loss of liver cells the meshes of the reticulum framework are narrowed and partly collapsed. Wilder's reticulum stain. (Branching bile ducts from this case are shown in Fig. 41; a photomicrograph of the spleen, in Fig. 57.) × 330.

Fig. 20. Case 76. Duration of disease, 96 days. The lobular reticulum is intact and, in general, has a normal pattern, although its meshes are somewhat distorted. The section is taken from the atrophic left lobe of the liver shown in Figure 12. (For changes in the brain of this case see Figs. 67, 68, 69 and 76.) × 170.



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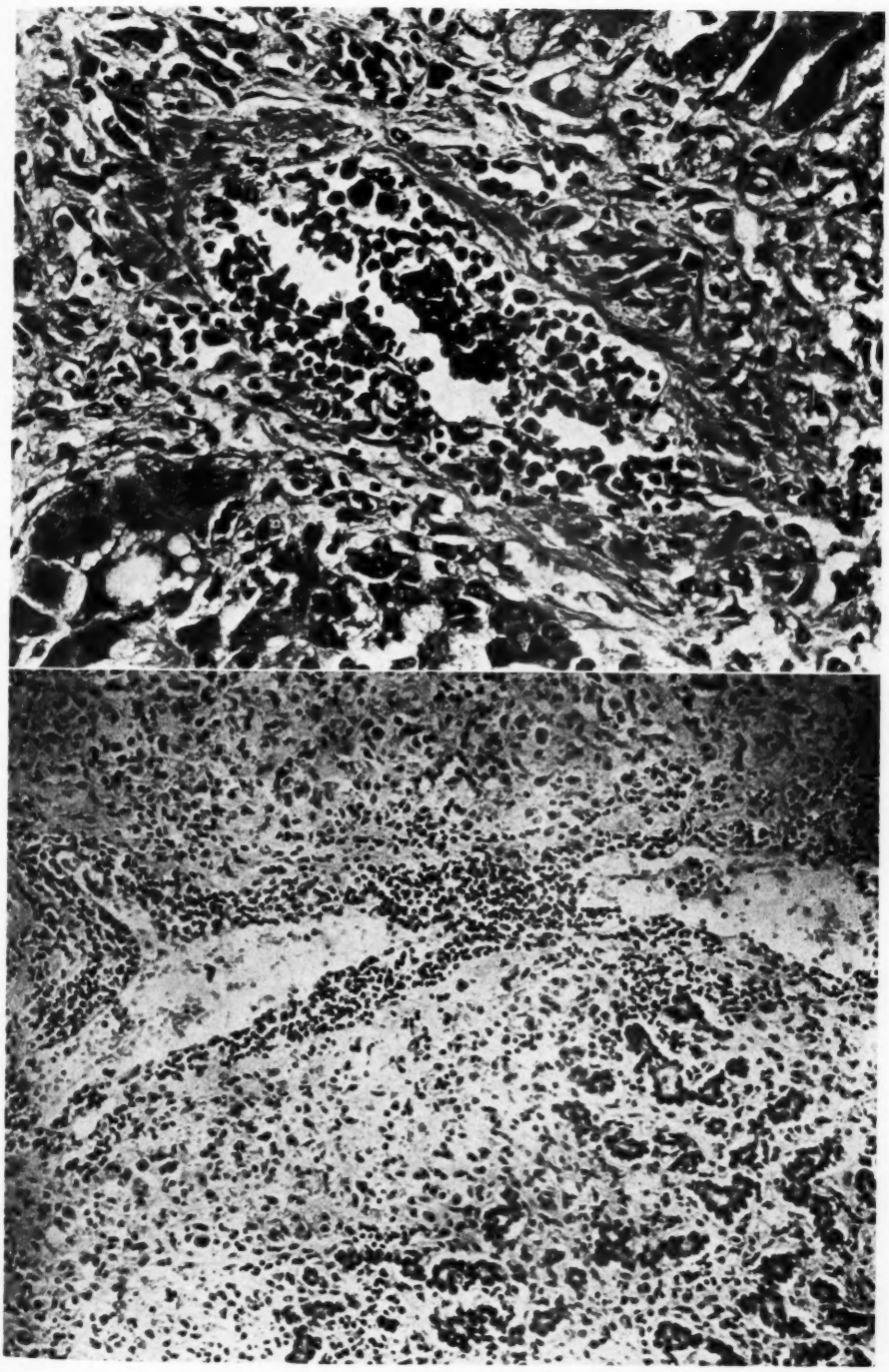
Pathology of Fatal Epidemic Hepatitis

PLATE 98

FIG. 21. Case 4. Duration of disease, 10 days. A central lobular vein has a greatly thickened wall which appears hyalinized. The subendothelial layer of the vein is infiltrated with cells of the same types as shown in Figure 16; the endothelial lining is intact. (See Figs. 13, 16, 24, 25, 26 and 39 for other photomicrographs of liver from same case.) × 500.

FIG. 22. Case 79. Duration of hepatitis, 57 days. A sublobular vein shows marked endophlebitis. (See Fig. 17 for appearance of the surrounding tissues.) × 175.



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Pathology of Fatal Epidemic Hepatitis

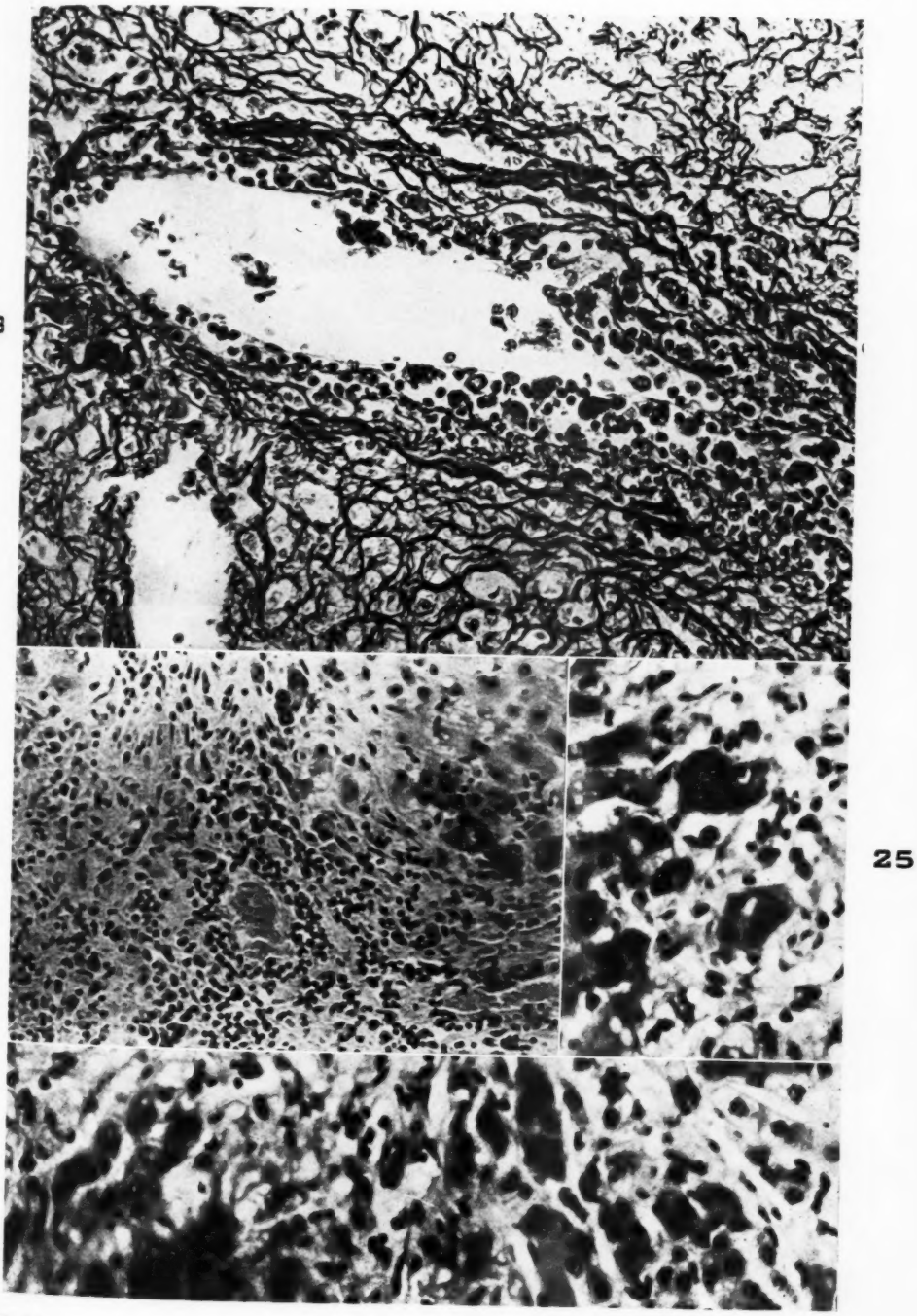
PLATE 99

FIG. 23. Case 79. Duration of disease, 57 days. A sublobular vein stained by Wilder's reticulum stain shows that the endothelium of the intima is intact. The subendothelial layer is infiltrated with numerous cells, some of which are pigmented macrophages. The walls of the vein have a loose structure. × 500.

FIG. 24. Case 4. Duration of disease, 10 days. Early multiplication of liver cells is shown adjacent to a region from which the parenchyma has disappeared. The regenerating cells have closely aggregated, deeply staining large nuclei. The stroma of the central part of the lobule is infiltrated with wandering cells, details of which are shown in Figure 16. The central lobular vein exhibits marked endophlebitis. (See Fig. 21 for details.) × 110.

FIG. 25. Case 4. Duration of disease, 10 days. The partly broken columns of liver cells show early regeneration, as indicated by numerous closely placed hyperchromatic nuclei; many cells are binucleated. × 330.

FIG. 26. Another field from the section shown in Figure 25. × 330.



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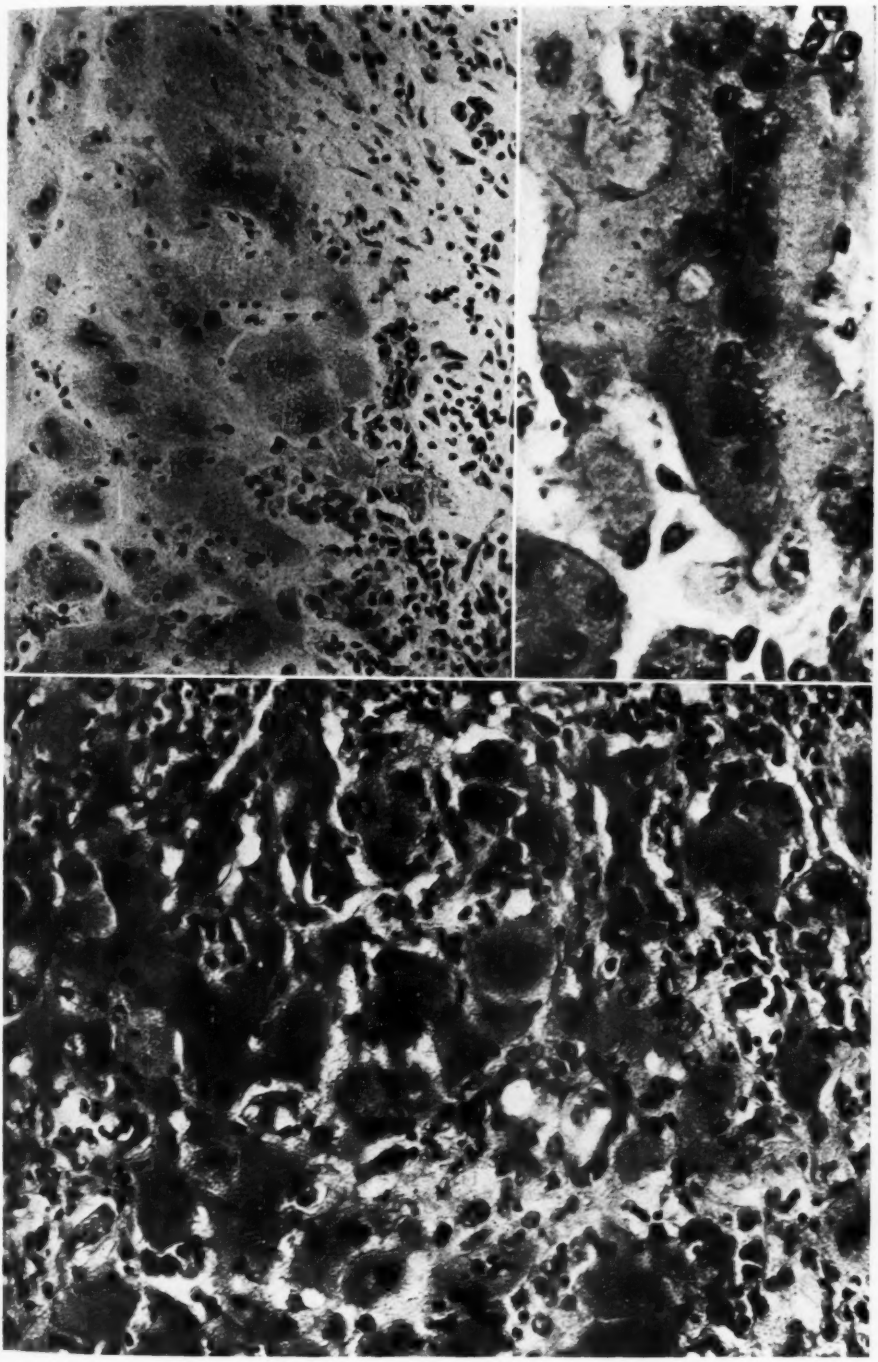
Pathology of Fatal Epidemic Hepatitis

Plate 100

Fig. 27. Case 8. Duration of disease, 43 days. A large multinucleated liver cell from an area of regeneration. The section is taken from the nodular areas of regeneration shown grossly in Figure 3. × 300.

Fig. 28. Case 8. Duration of disease, 43 days. A multinucleated liver "giant" cell at higher magnification than in Figure 27. The cytoplasm of the cell contains granules of bile pigment. × 700.

Fig. 29. Case 53. Duration of disease, 34 days. A group of liver cells, which are arranged in such a disorderly manner as to simulate neoplasia. A number of cells are multinucleated. The cytoplasm of most cells has a granular appearance because of the presence of bile. × 200.



27 28 29

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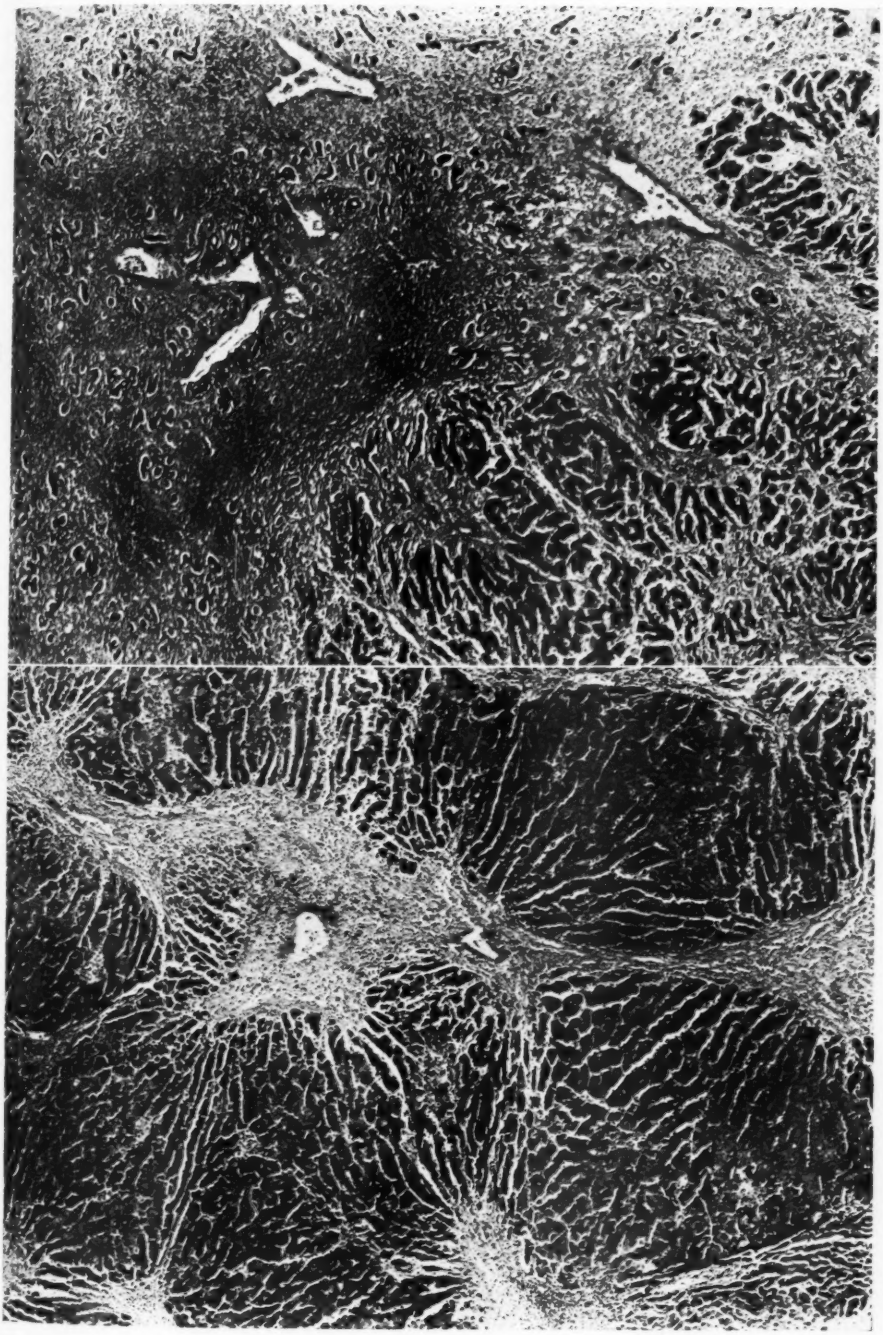
PLATE 101

FIG. 30. Case 24. Duration of disease, 26 days. The photomicrograph shows the edge of a region of regenerative hyperplasia. The section was taken from the liver illustrated in Figure 1. The hyperplastic parenchyma has no orderly arrangement. Where complete destruction has occurred, the lobular outlines are still indicated by proliferating bile ducts. The empty stroma stains darkly because of excessive blood in the sinusoids. × 35.

FIG. 31. Case 81. Duration of disease, 19 days. The section has been taken from one of the yellow patches of regenerating "lobules" shown in Figure 2. The newly formed "lobules" are large, have an atypical structure and are noticeably ischemic. (Fig. 4 shows the microscopic appearance of the liver from this case in the regions of "red atrophy"; here the sinusoids are prominently distended.) × 50.



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Pathology of Fatal Epidemic Hepatitis

PLATE 102

FIG. 32. Case 84. Duration of disease, 93 days. Section from nodular areas shown grossly in Figure 11. Large, confluent patches of parenchyma are seen; there is no arrangement into definite lobules. (See Figs. 37 and 46 for higher magnification; the changes in the kidney from this case are shown in Figs. 60 and 61; the brain changes in Figs. 71 and 72.) × 35.

FIG. 33. Case 86. Duration of disease, 62 days. The reticulum framework and the columns of liver cells converge in normal manner toward the central lobular vein. (See Fig. 70 for changes in the brain.) × 200.

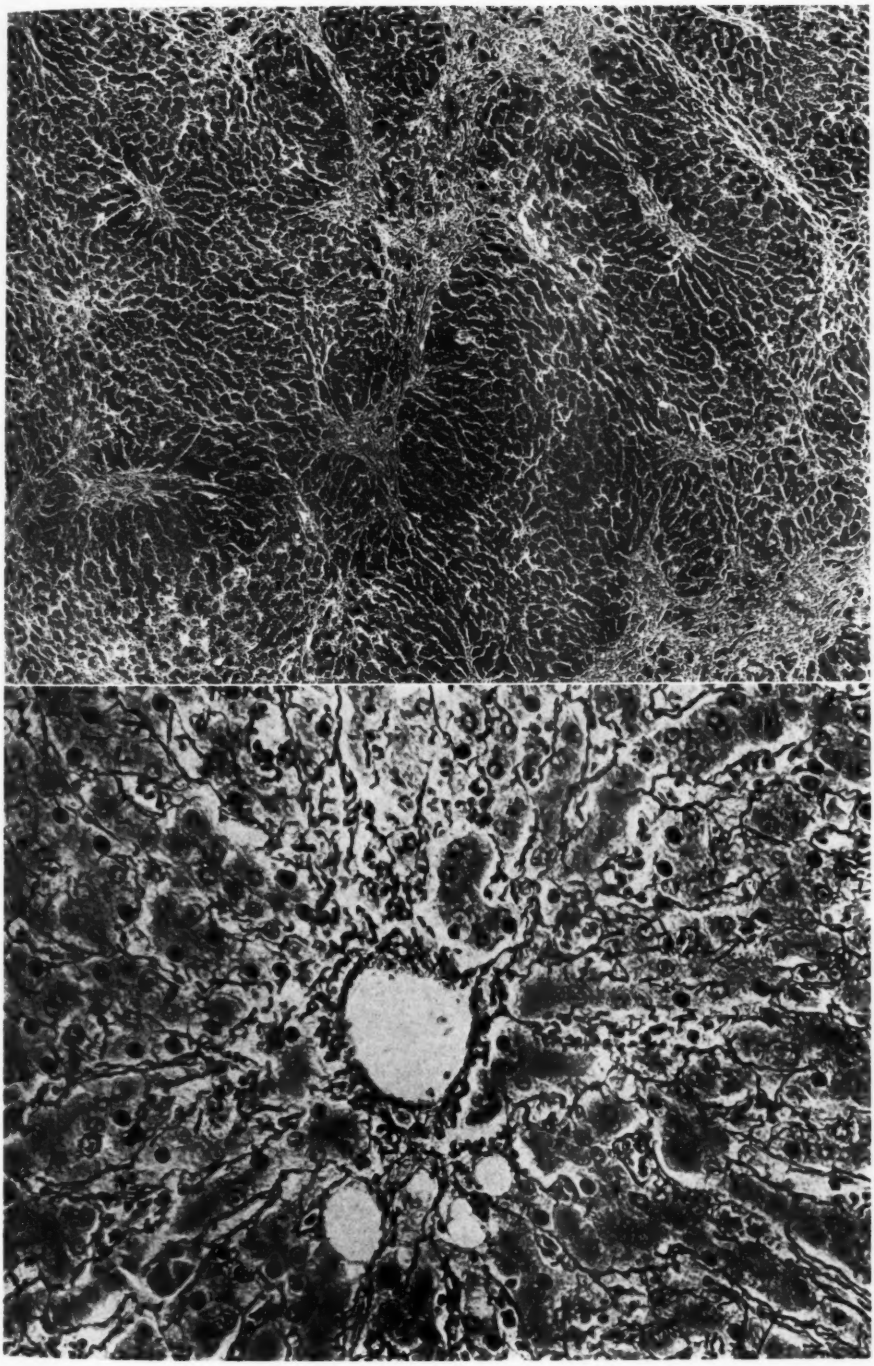
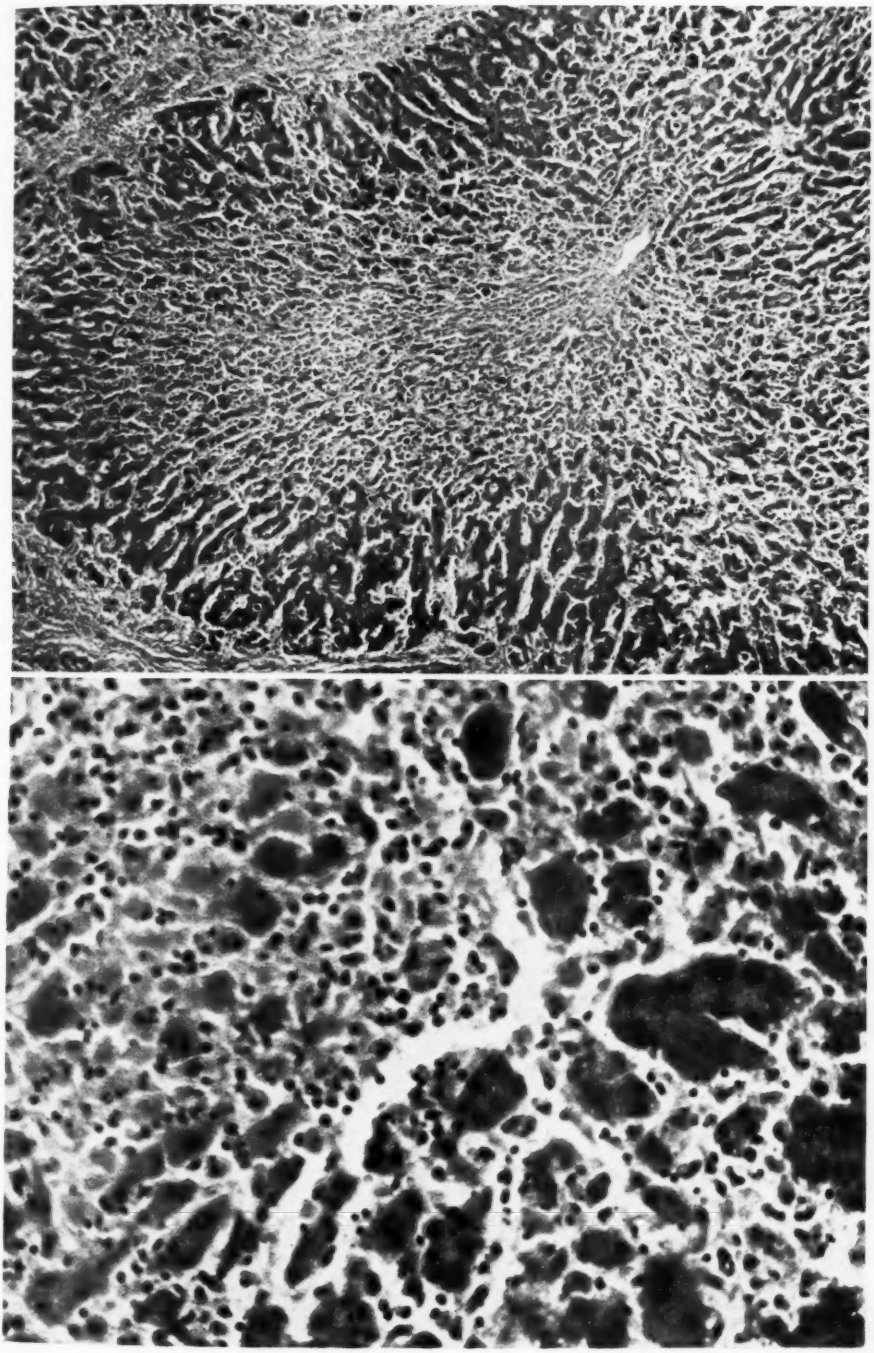


PLATE 103

FIG. 34. Case 93. Duration of disease, 76 days. A large hyperplastic "lobule," the central region of which is made up of atrophic and more or less disunited liver cells, whereas the cells at the periphery form columns. (Fig. 18, from the same case, shows a region of complete destruction of hepatic parenchyma, with preservation of the bile ducts.) × 60.

FIG. 35. Case 58. Duration of disease, 40 days. Secondary degeneration in an area of regenerative hyperplasia. Because of marked ischemia and perhaps other factors, the newly formed cells are undergoing degenerative changes. (The large size and hyperchromatic nuclei of the hyperplastic liver cells are noteworthy features.) A marked lymphocytic and polymorphonuclear reaction is seen in the stroma. × 200.



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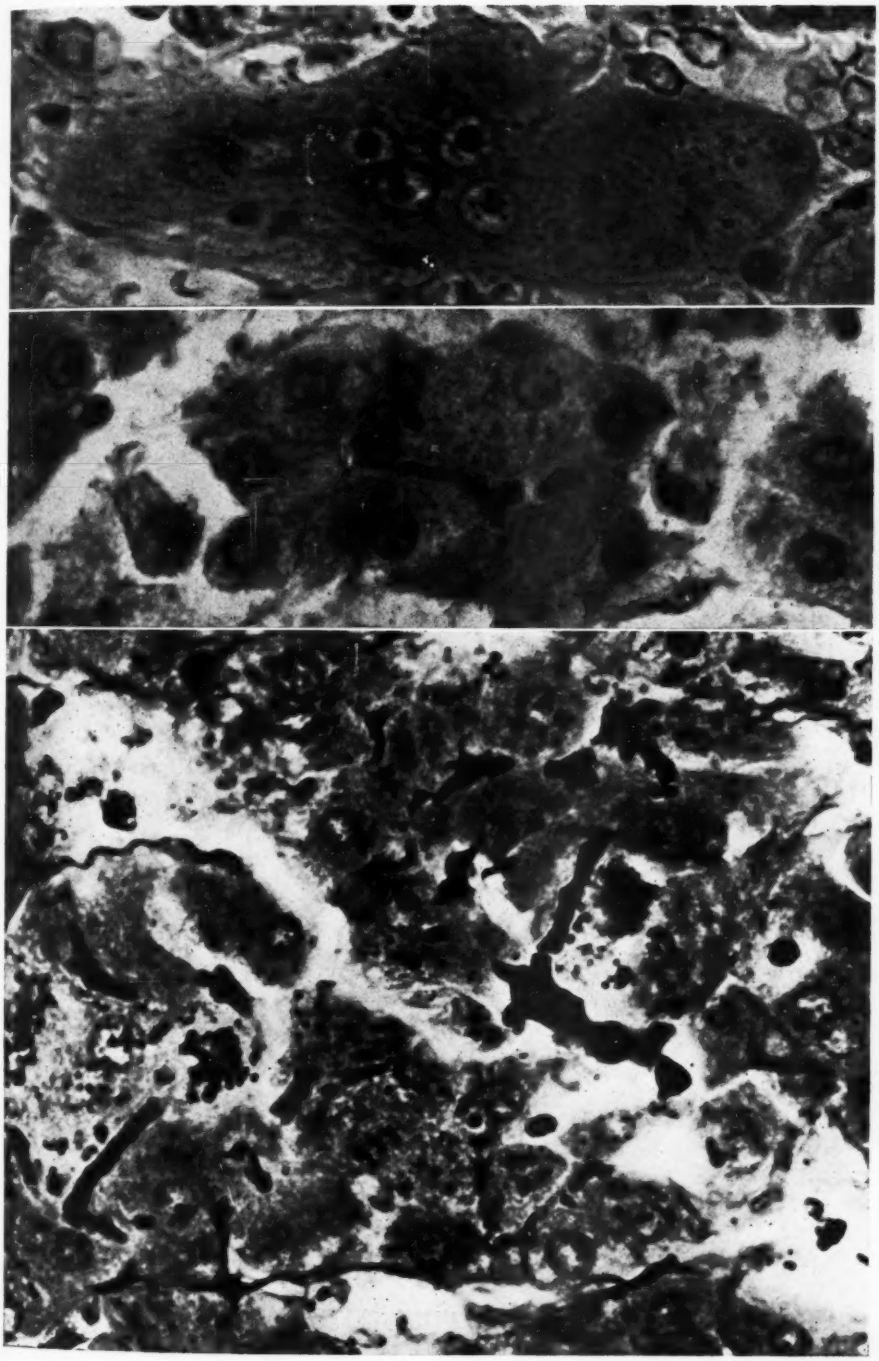
Pathology of Fatal Epidemic Hepatitis

PLATE 104

FIG. 36. Case 66. Duration of disease, 24 days. A multinucleated "giant" liver cell, the nuclei of which have prominent nucleoli. The latter must not be mistaken for intranuclear inclusion bodies. × 1000.

FIG. 37. Case 84. Duration of disease, 93 days. The bile canaliculus of an hepatic column is distended with a plug of inspissated bile. The liver cells are well preserved. (The gross appearance of this liver is shown in Fig. 11; for general structural changes see Figs. 5, 32 and 46; for lesions in the kidney, Figs. 60 and 61, and for brain changes, Figs. 71 and 72.) × 1000.

FIG. 38. Case 117. Duration of disease, 240 days. Section is stained with Wilder's reticulum stain. Near the center of the figure is seen a thick branching bile "thrombus"; several other bile "thrombi" lie within the nearby canaliculi; the individual liver cells contain coarse granules of bile. × 850.



Lucké

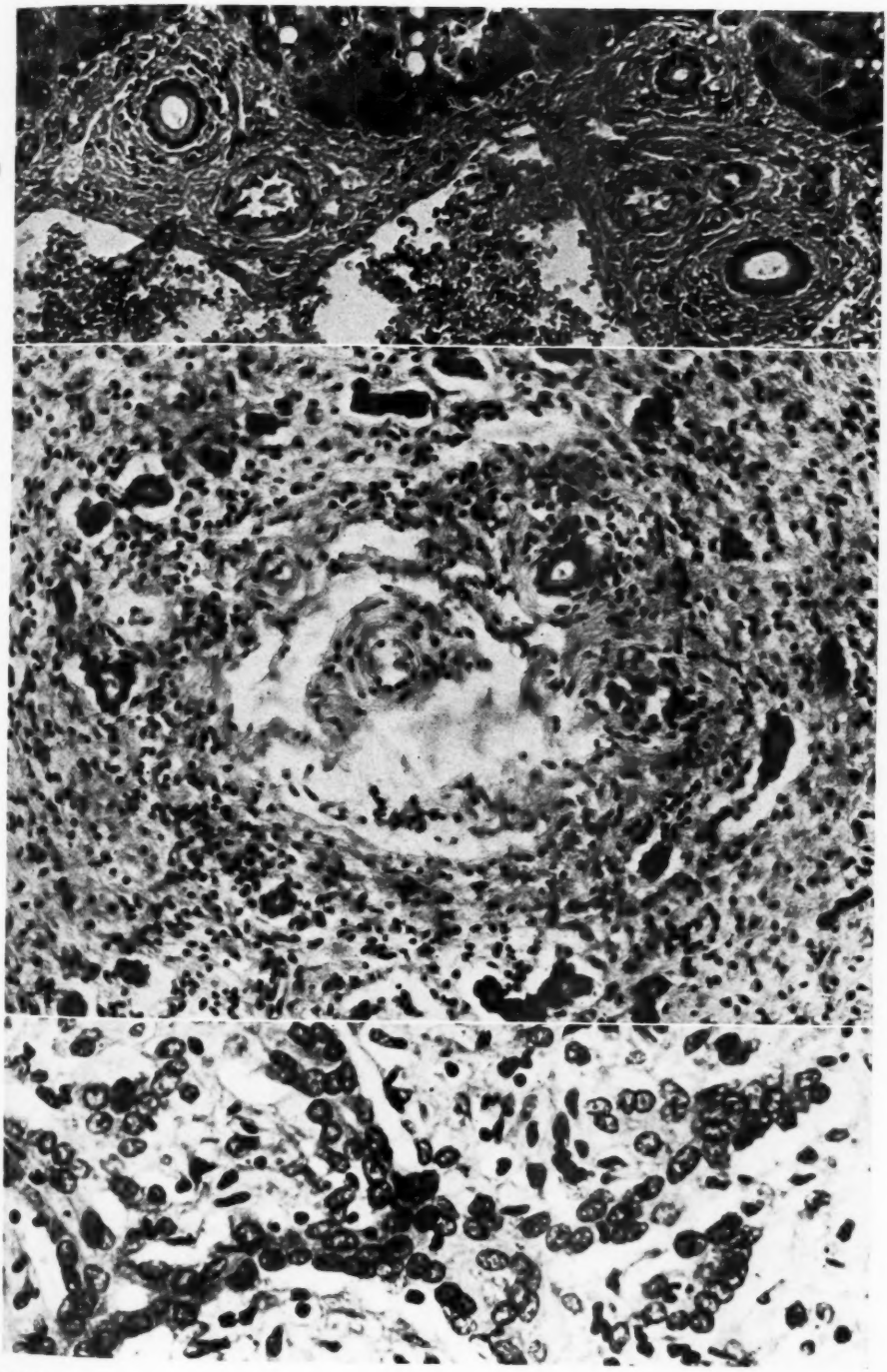
Pathology of Fatal Epidemic Hepatitis

PLATE 105

FIG. 39. Case 4. Duration of disease, 10 days. A portal triad with several large interlobular bile ducts having normal structure. The branches of the portal vein and hepatic artery likewise are normal. × 100.

FIG. 40. Case 15. Duration of disease, 14 days. Early budding of biliary ducts. The portal stroma is richly cellular. (Fig. 15 shows the extent of parenchymatous destruction in nearby parts of the liver of the same case.) × 125.

FIG. 41. Case 80. Duration of disease, 30 days. Proliferating bile duct at the periphery of a lobule. The lining cells have closely placed oval nuclei and scanty cytoplasm. × 350.



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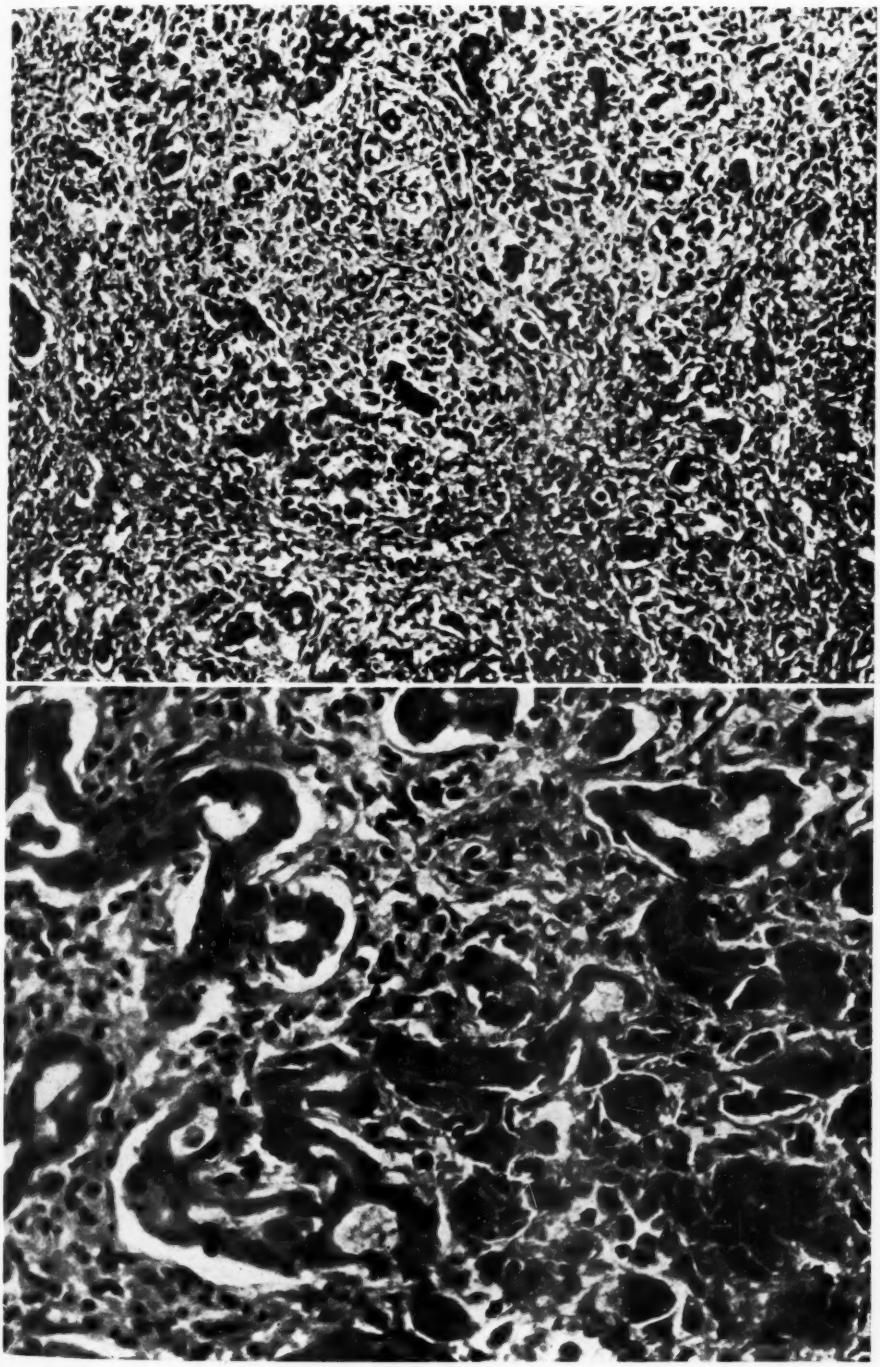
Pathology of Fatal Epidemic Hepatitis

Plate 106

Fig. 42. Case 117. Duration of disease, 240 days. In regions in which the hepatic cells had been destroyed completely, proliferated small bile ducts remain in a richly vascular stroma. × 150.

Fig. 43. Case 46. Duration of disease, 15 days. Tubular structures at the periphery of a lobule; some parts of the tubules are lined by cells resembling bile duct epithelium, whereas other parts are lined by elements resembling liver cells. × 400.



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Pathology of Fatal Epidemic Hepatitis

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PLATE 107

FIG. 44. Case 92. Duration of disease, 69 days. Two large clumps of bile, one lying in a widely dilated intracolumnar canaliculus, the other in a tubule apparently lined by biliary epithelium. (See Fig. 9 for gross appearance of liver, and Fig. 59 for changes in kidney.) × 550.

FIG. 45. Case 113. Duration of disease, 154 days. The lumina of tubular structures at the periphery of lobular remnants contain clumps of bile. It is impossible to be sure whether these tubules are composed of biliary epithelium or of liver cells. × 350.

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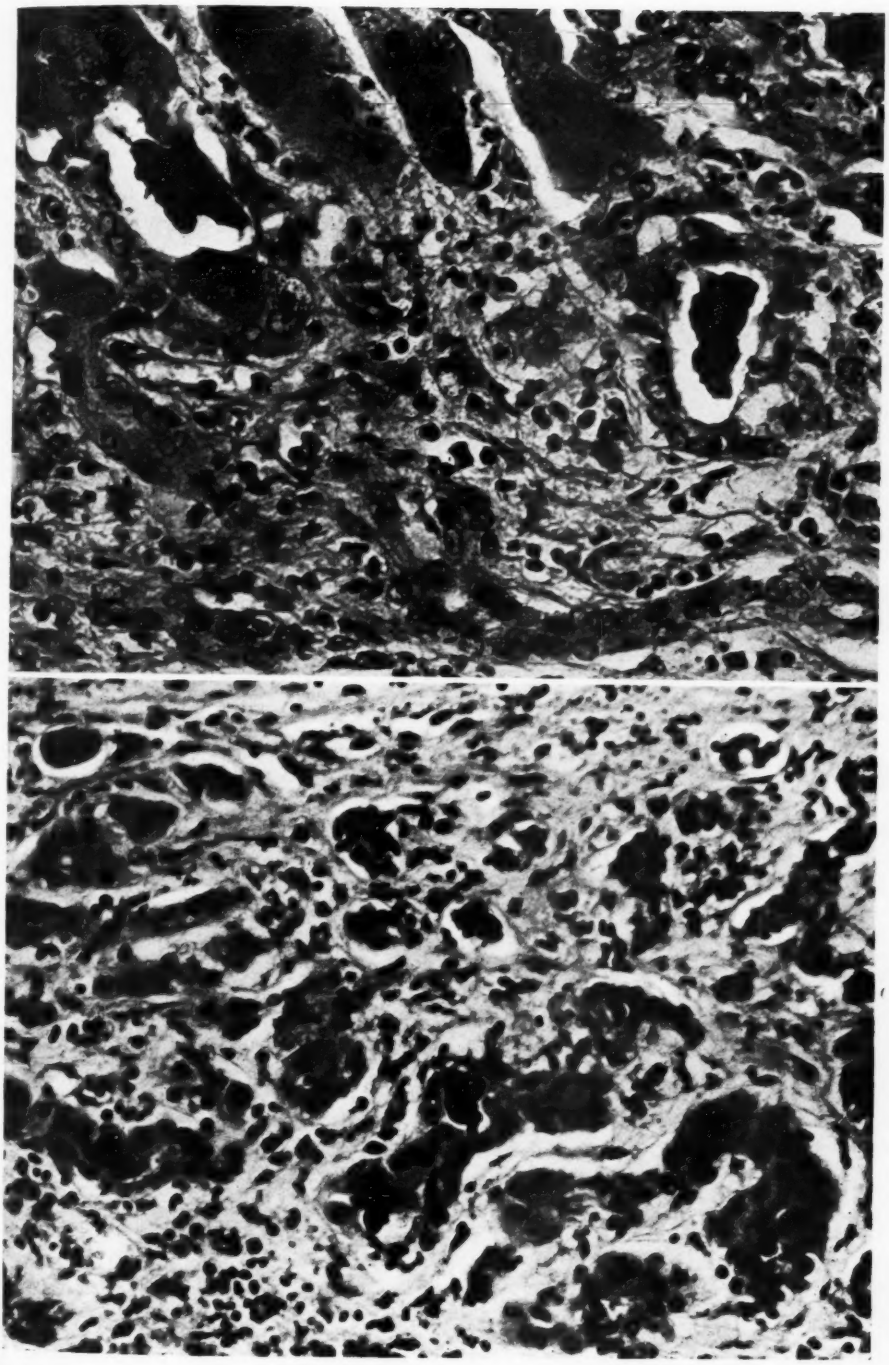
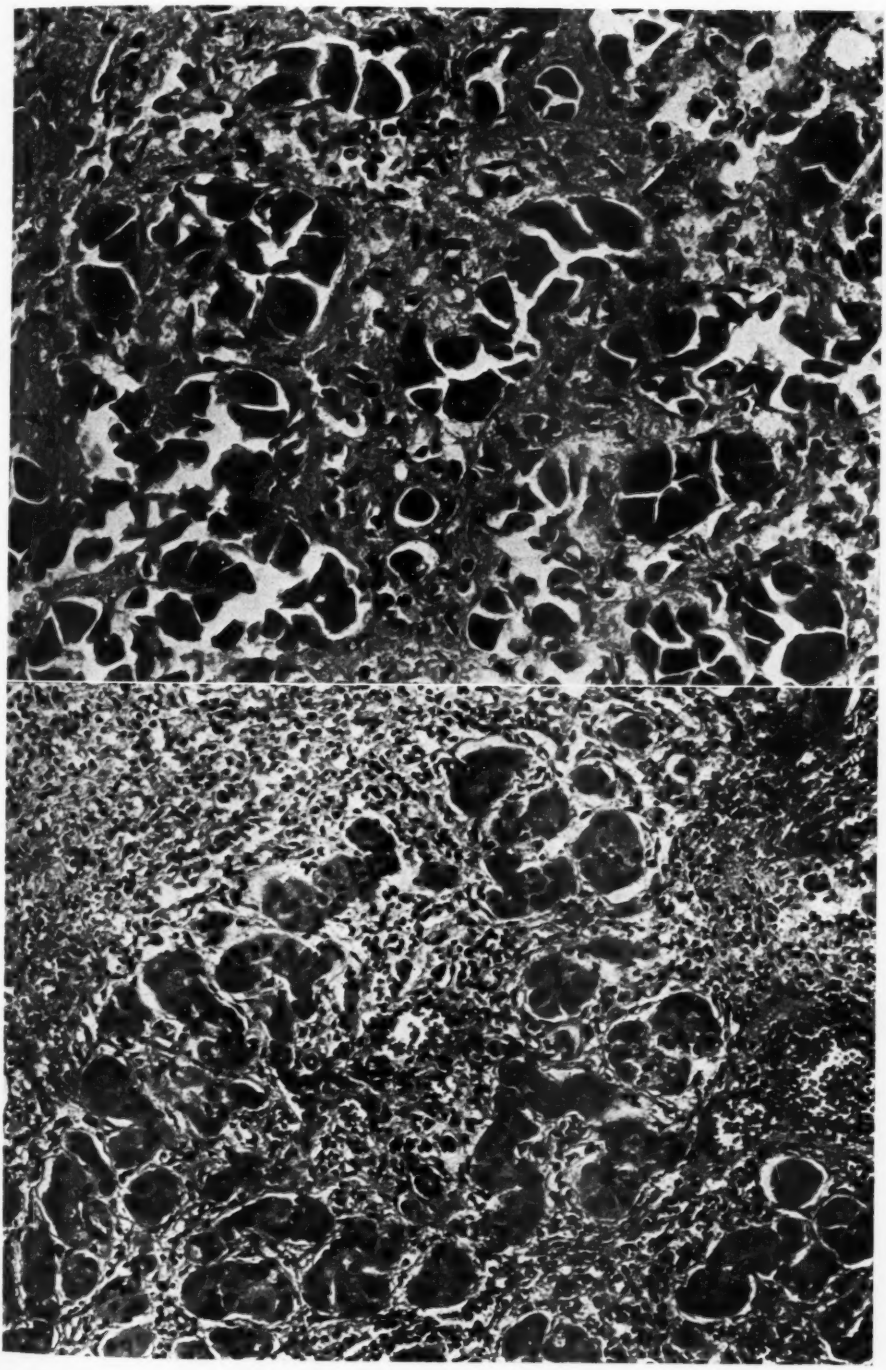


Lucké Pathology of Fatal Epidemic Hepatitis

PLATE 108

FIG. 46. Case 84. Duration of disease, 93 days. The cells of the regenerated tubules closely resemble liver cells. Whether these cells are derived from biliary epithelium or from pre-existing liver cells is debatable. (See also Figs. 5, 11, 32, 37, 60, 61, 71 and 72 for photographs of other lesions in this case.) × 550.

FIG. 47. Case 104. Duration of disease, 98 days. A cluster of tubules lined by liver cells lies at the periphery of a lobule. The location and appearance of the tubules suggest that they have been derived from bile ducts. (See also Fig. 7 from the same case which shows a branching bile duct connected with regenerated columns of liver cells.) × 300.



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Pathology of Fatal Epidemic Hepatitis

PLATE 109

FIG. 48. Case 22. Duration of hepatitis, 17 days. Cecum. The tissue is extremely edematous; the mucosa is thrown into thick folds. (See Fig. 51 for microscopic appearance.)

FIG. 49. Case 11. Duration of hepatitis, 64 days. Transverse sections of lower ileum showing edematous thickening of wall and of mesenteric attachment.

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Pathology of Fatal Epidemic Hepatitis

Plate 110

Fig. 50. Case 43. Duration of hepatitis, 20 days. Low-power view of colon to show marked inflammatory edema of submucosa. The mucosa is intact. (See Fig. 52 for details of cellular reaction.) × 10.

Fig. 51. Case 22. Duration of disease, 17 days. Low-power view of cecum shown in Figure 48. There is marked phlegmonous inflammation which is especially pronounced in the submucosa. The mucosa is preserved. × 10.

50

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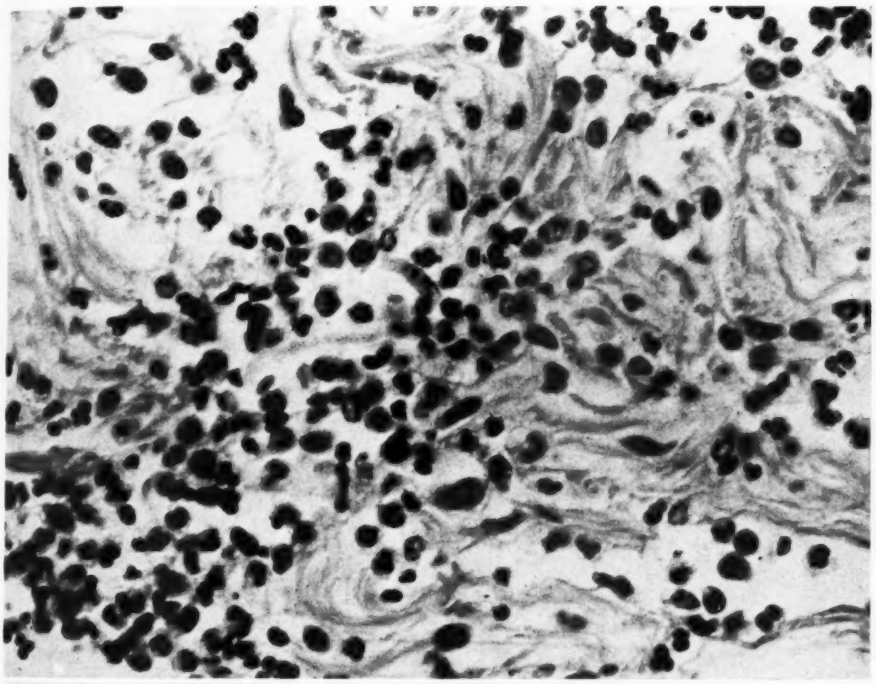
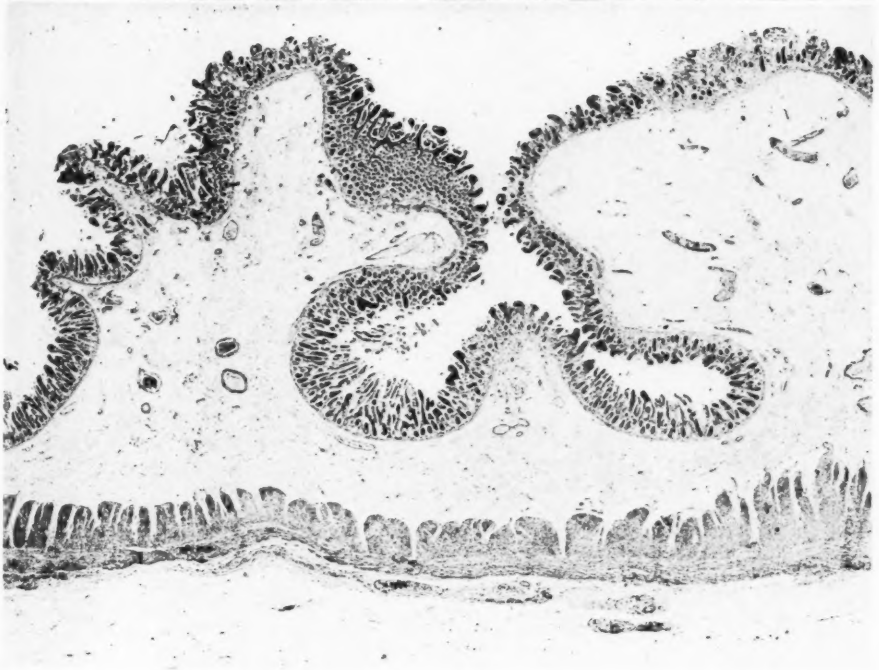
Pathology of Fatal Epidemic Hepatitis

PLATE 111

FIG. 52. Case 43. Duration of hepatitis, 20 days. Details of inflammatory edema of wall of cecum shown in Figure 50. The tissue is widely distended and everywhere invaded by polymorphonuclear leukocytes and macrophages. × 550.

FIG. 53. Case 94. Duration of hepatitis, 50 days. A low-power view of ileum showing marked distention of submucosa by edema. No inflammatory reaction is present. The mucosa is preserved. × 12.





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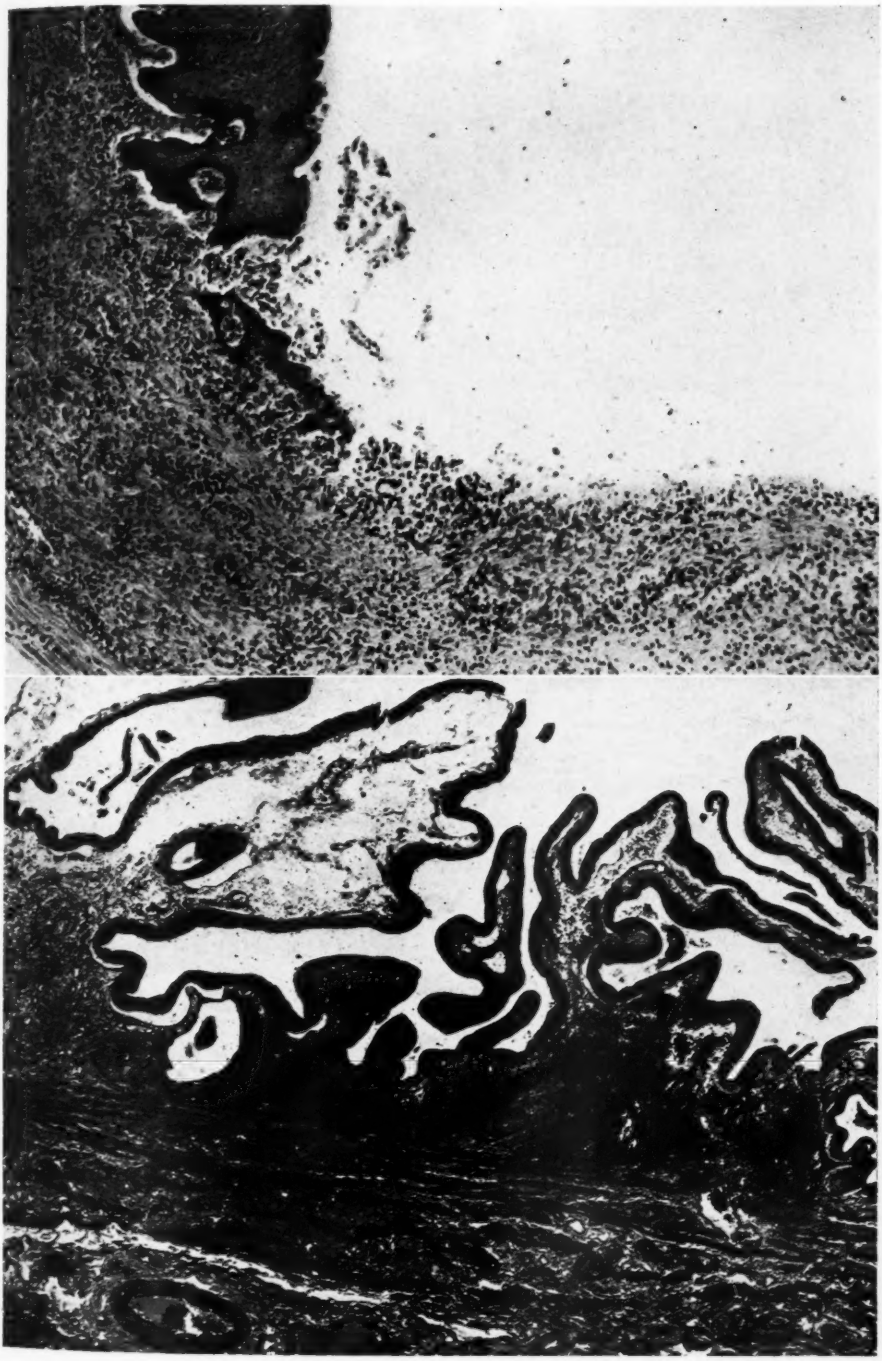
Pathology of Fatal Epidemic Hepatitis

Plate 112

Fig. 54. Case 8. Duration of hepatitis, 43 days. Ulceration of lower portion of esophagus with inflammatory reaction extending into deeper layers. (A gross photograph of the liver from this case is shown in Fig. 3, and the microscopic appearance of the liver in Figs. 27 and 28.) × 75.

Fig. 55. Case 39. Duration of hepatitis, 34 days. Gallbladder showing marked edema and hemorrhages in mucosa. × 25.



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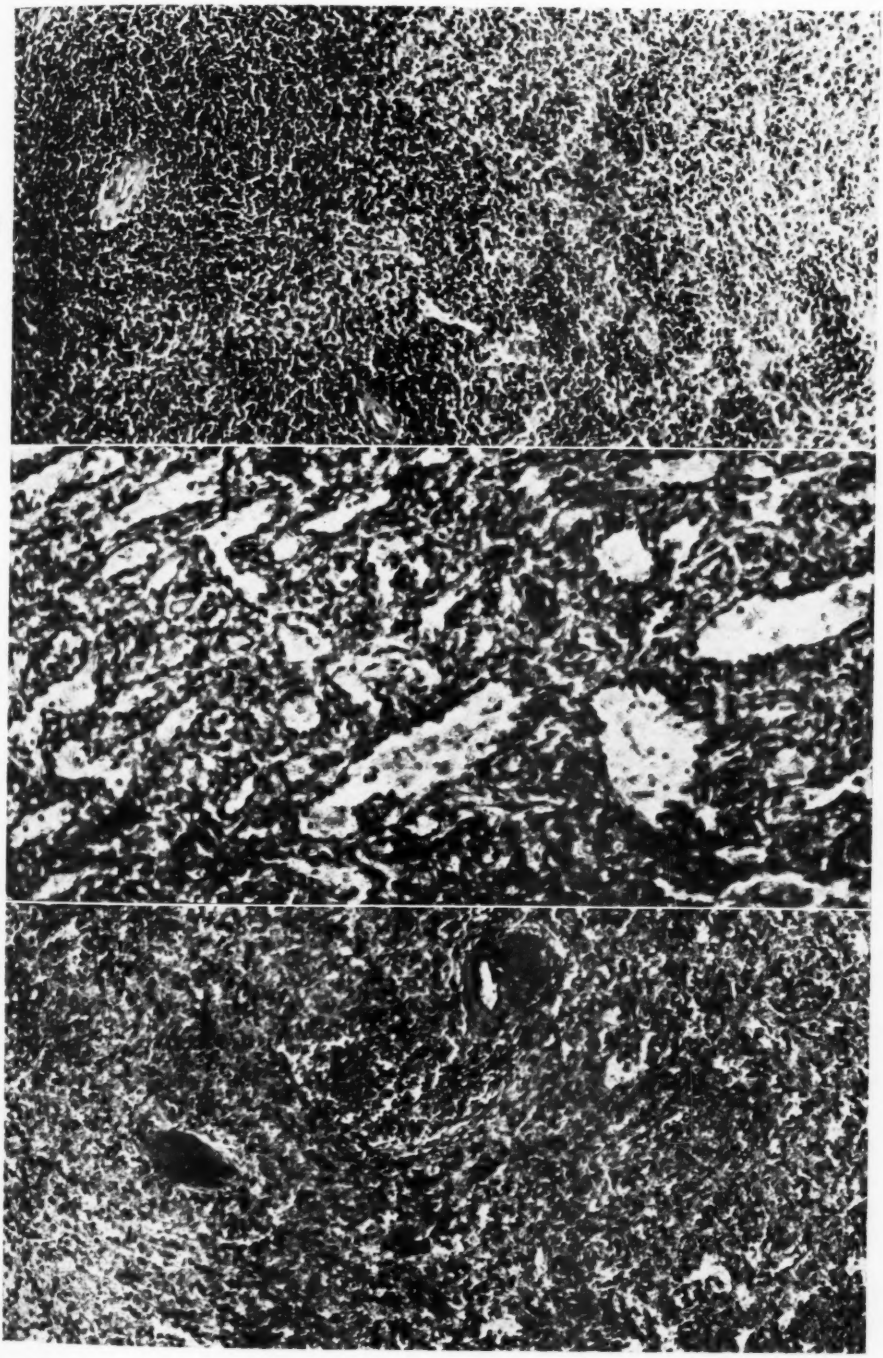
Pathology of Fatal Epidemic Hepatitis

PLATE 113

FIG. 56. Case 43. Duration of hepatitis, 20 days. Spleen. Hyperplasia of a follicle and of lymphoid tissue in pulp. × 50.

FIG. 57. Case 80. Duration of hepatitis, 30 days. Spleen. Marked engorgement of sinusoids, the walls of which have a rigid appearance. (See Figs. 19 and 41 for changes in the liver.) × 80.

FIG. 58. Case 108. Duration of hepatitis, 39 days. Spleen, showing depletion of lymphoid tissue; the follicle is atrophic. The sinusoids have rigid walls. × 50.



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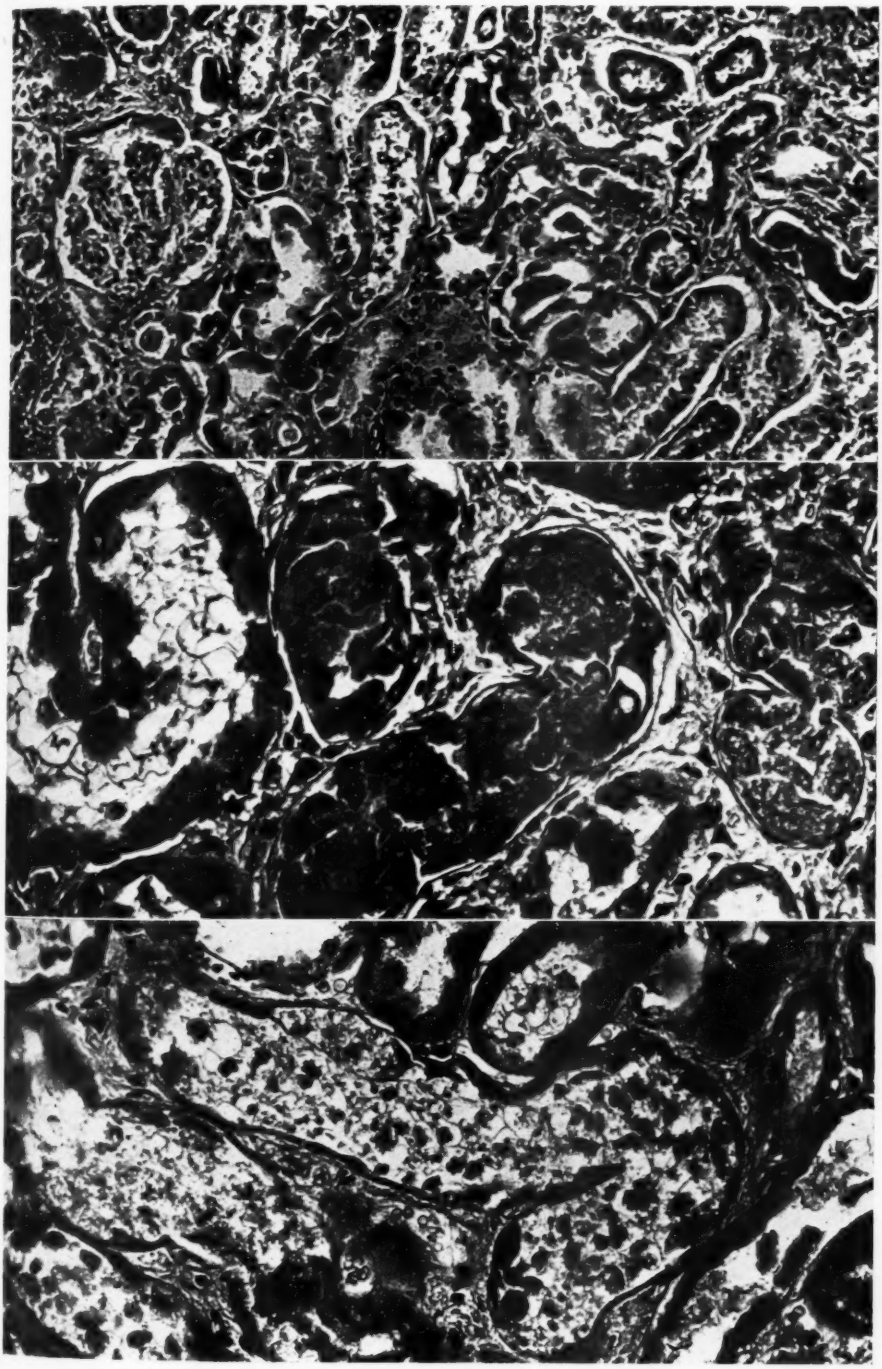
Pathology of Fatal Epidemic Hepatitis

PLATE 114

FIG. 59. Case 92. Duration of hepatitis, 67 days. Kidney, showing an intact glomerulus, and many tubules with bile casts. (A gross photograph of the liver of this case is shown in Fig. 9, and a photomicrograph in Fig. 44.) × 125.

FIG. 60. Case 84. Duration of hepatitis, 93 days. Several tubules are blocked with necrotic cells, beneath which is seen flat epithelium indicative of early regeneration. × 550.

FIG. 61. From the same case as Figure 60. In this tubule the necrotic cells have been removed. The lining epithelium is flat and has the appearance characteristic of early regeneration. × 375.



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Pathology of Fatal Epidemic Hepatitis

PLATE 115

FIG. 62. Case 24. Duration of hepatitis, 36 days. Heart showing petechiae of epicardium and ecchymosis beneath the endocardium of the interventricular septum, near the bases of the aortic cusps.

FIG. 63. Colon with mesocolon and epiploic appendages, from case 24. An extensive hemorrhage is seen in the mesocolon.

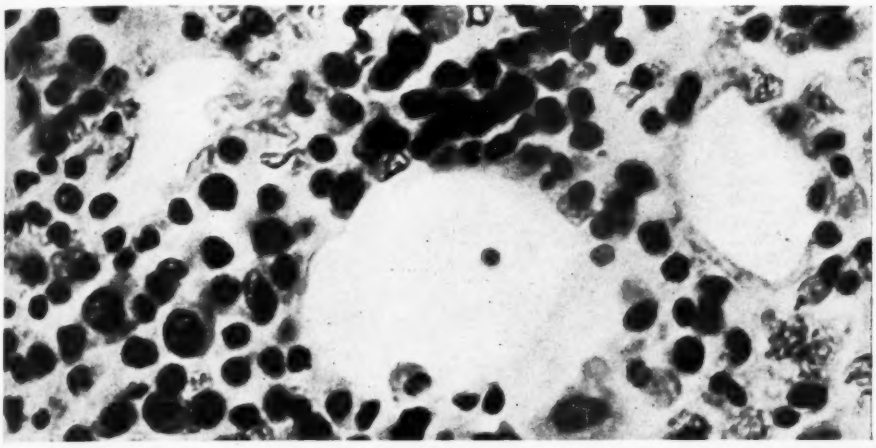
Lucké Pathology of Fatal Epidemic Hepatitis

PLATE 116

FIG. 64. Case 94. Duration of hepatitis, 50 days. Bone marrow, showing a moderate degree of hyperplasia, particularly of the red cell series. × 450.

FIG. 65. Case 71. Duration of hepatitis, 49 days. Bone marrow, showing diffuse hyperplasia, particularly of the myeloid elements. × 450.

FIG. 66. Case 67. Duration of disease, 19 days. Bone marrow, showing marked hyperplasia, particularly of the red cell series. × 450.



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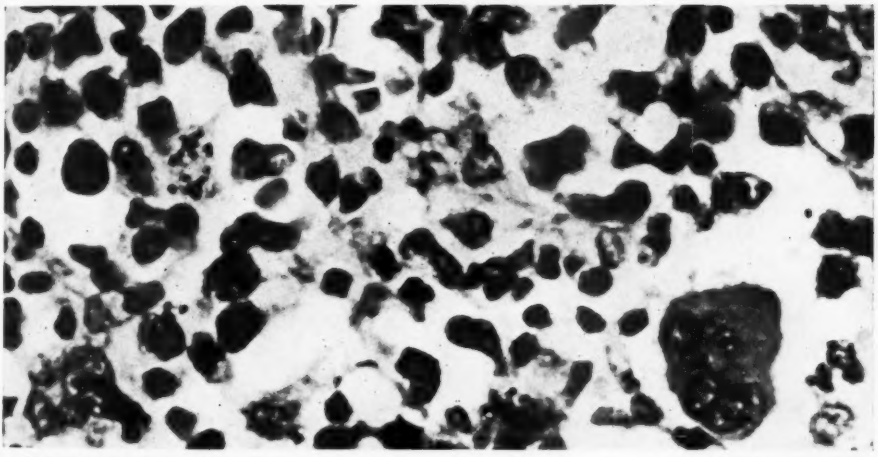

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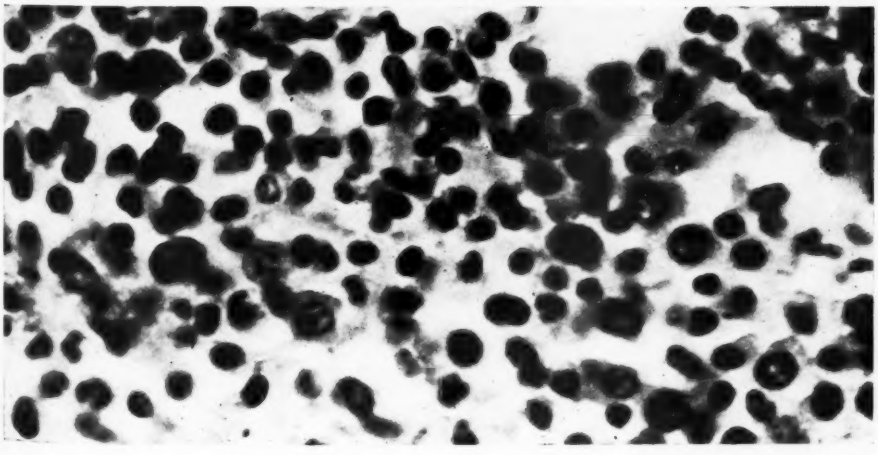

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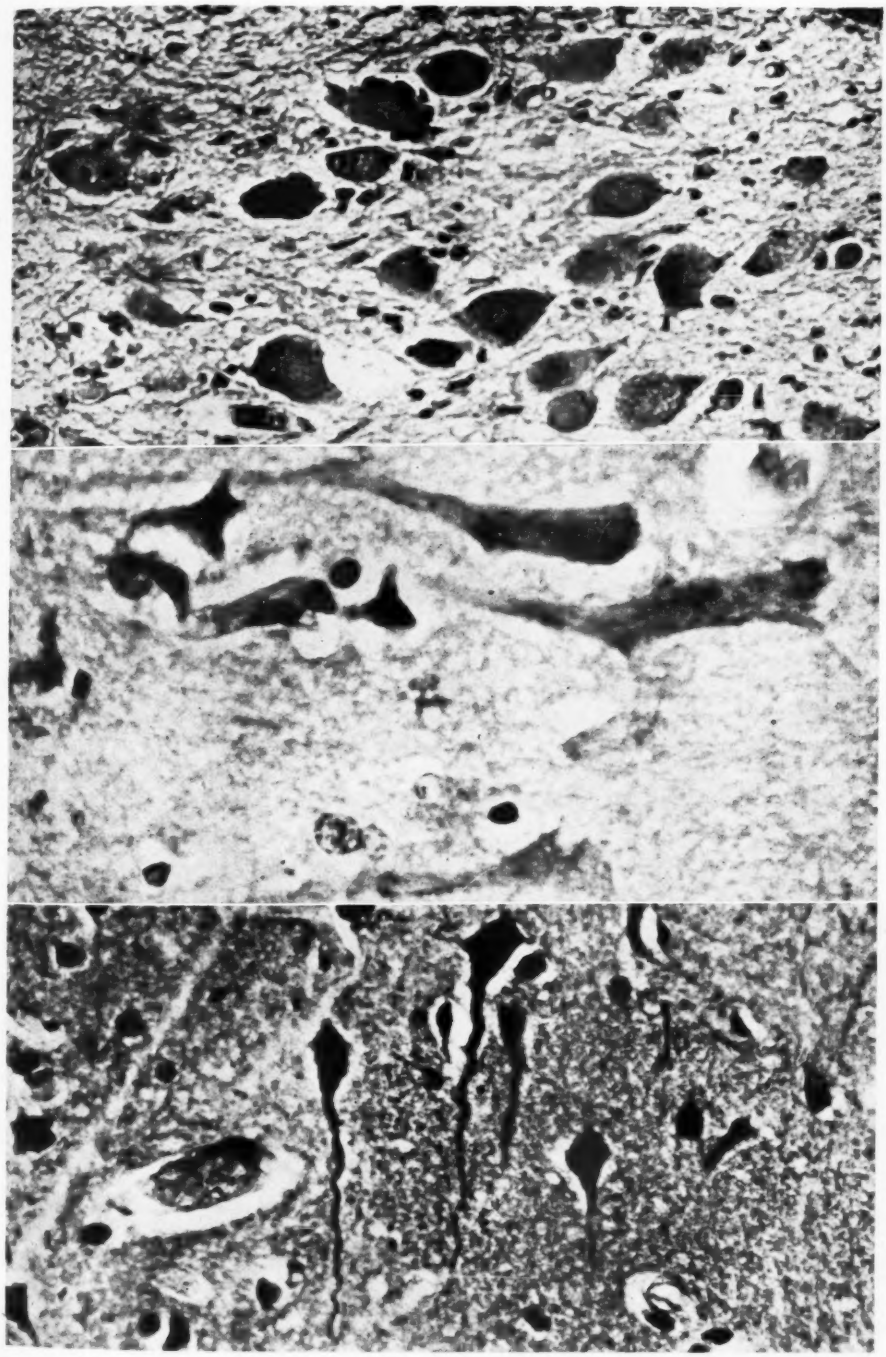
Pathology of Fatal Epidemic Hepatitis

PLATE 117

FIG. 67. Case 76. Duration of hepatitis, 96 days. Base of brain; section through nucleus basalis. The ganglion cells show various stages of disintegration. There is no glial reaction. (For other changes in the brain of this case see Figs. 68, 69 and 76; the gross appearance of the liver is shown in Fig. 12, and a photomicrograph in Fig. 20.) × 200.

FIG. 68. Case 76. Brain; third layer of cortex. The photomicrograph shows three large ganglion cells. The upper cell has a broad apical dendrite and a well preserved nucleus and nucleolus. In the middle cell the apical dendrite is broadened; the nucleus has almost disappeared; the chromatin has been largely dissolved. The lower cell has been converted into a "ghost." (See Fig. 67.) × 800.

FIG. 69. Case 76. Brain; section through midcortex. The ganglion cells have shrunken, densely staining bodies. The apical dendrites are prominent and tortuous. The change corresponds to "chronic" cell degeneration. (See Fig. 67.) × 680.



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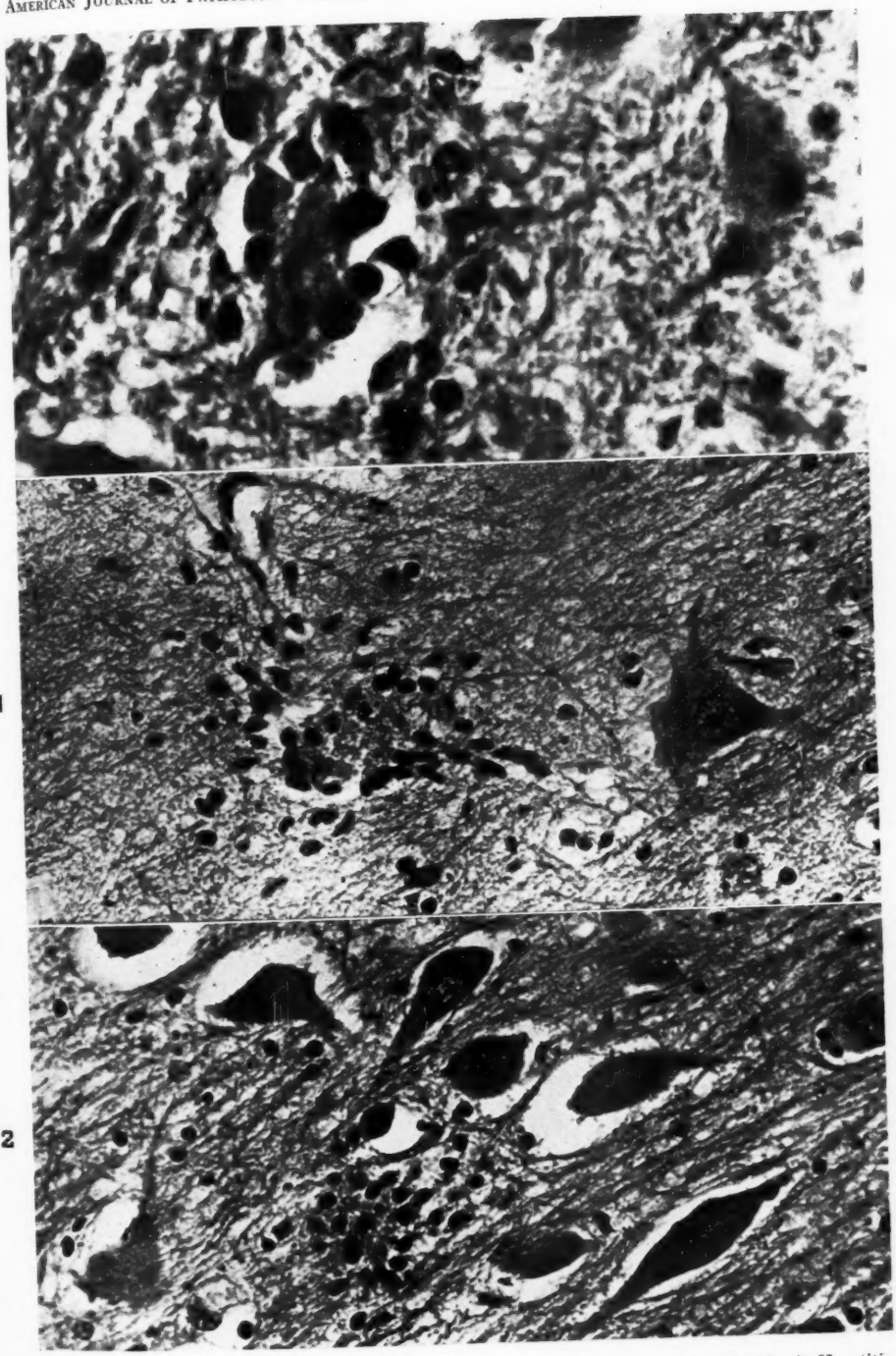
Pathology of Fatal Epidemic Hepatitis

PLATE 118

FIG. 70. Case 86. Duration of hepatitis, 86 days. Brain; section through medulla oblongata. Invasion of a disintegrated ganglion cell by glial elements (neuronophagia). × 1000.

FIG. 71. Case 84. Duration of hepatitis, 93 days. Brain; section through nucleus basalis. A disintegrated ganglion cell invaded by glial elements (neuronophagia). A nearby ganglion cell is intact. (Gross appearance of liver from this case is shown in Fig. 11, and photomicrographs in Figs. 5, 32, 37 and 46.) × 435.

FIG. 72. Case 84. Brain; section through nucleus basalis. A small glial nodule has replaced a destroyed ganglion cell. The ganglion cells shown in the photomicrograph are in varying stages of degeneration. × 435.



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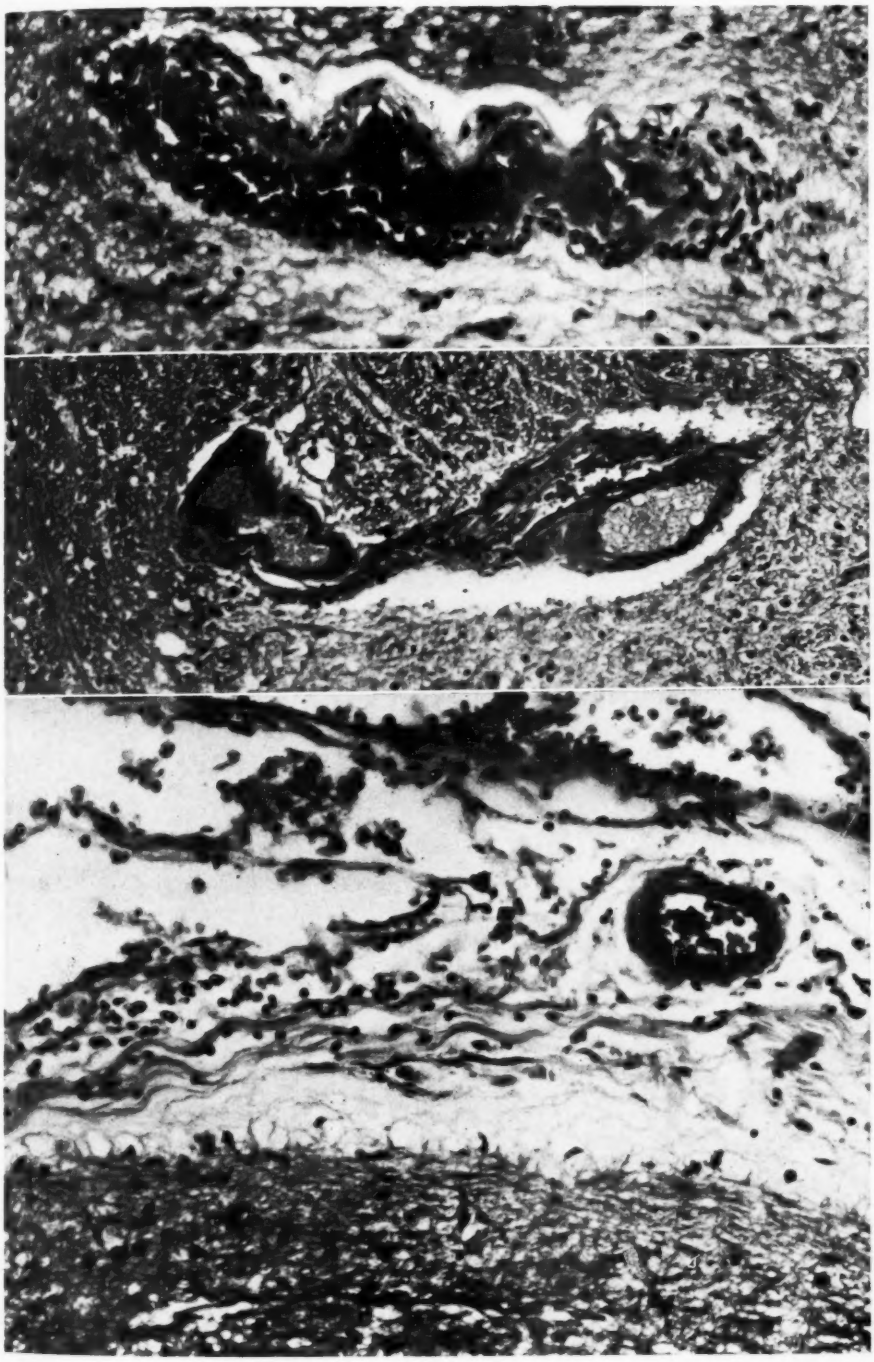
Pathology of Fatal Epidemic Hepatitis

PLATE 119

FIG. 73. Case 124. Duration of hepatitis, 37 days. Brain; periventricular system adjacent to thalamus with perivascular lymphocytic infiltration. The vessel involved is located beneath the ependyma of the third ventricle. (For reaction in meninges see Fig. 75; gross appearance of liver is shown in Fig. 10.) × 300.

FIG. 74. Case 78. Duration of hepatitis, 64 days. Tegmentum of pons. Slight perivascular lymphocytic infiltration. This represents the average degree of perivascular infiltration observed in the present series. × 175.

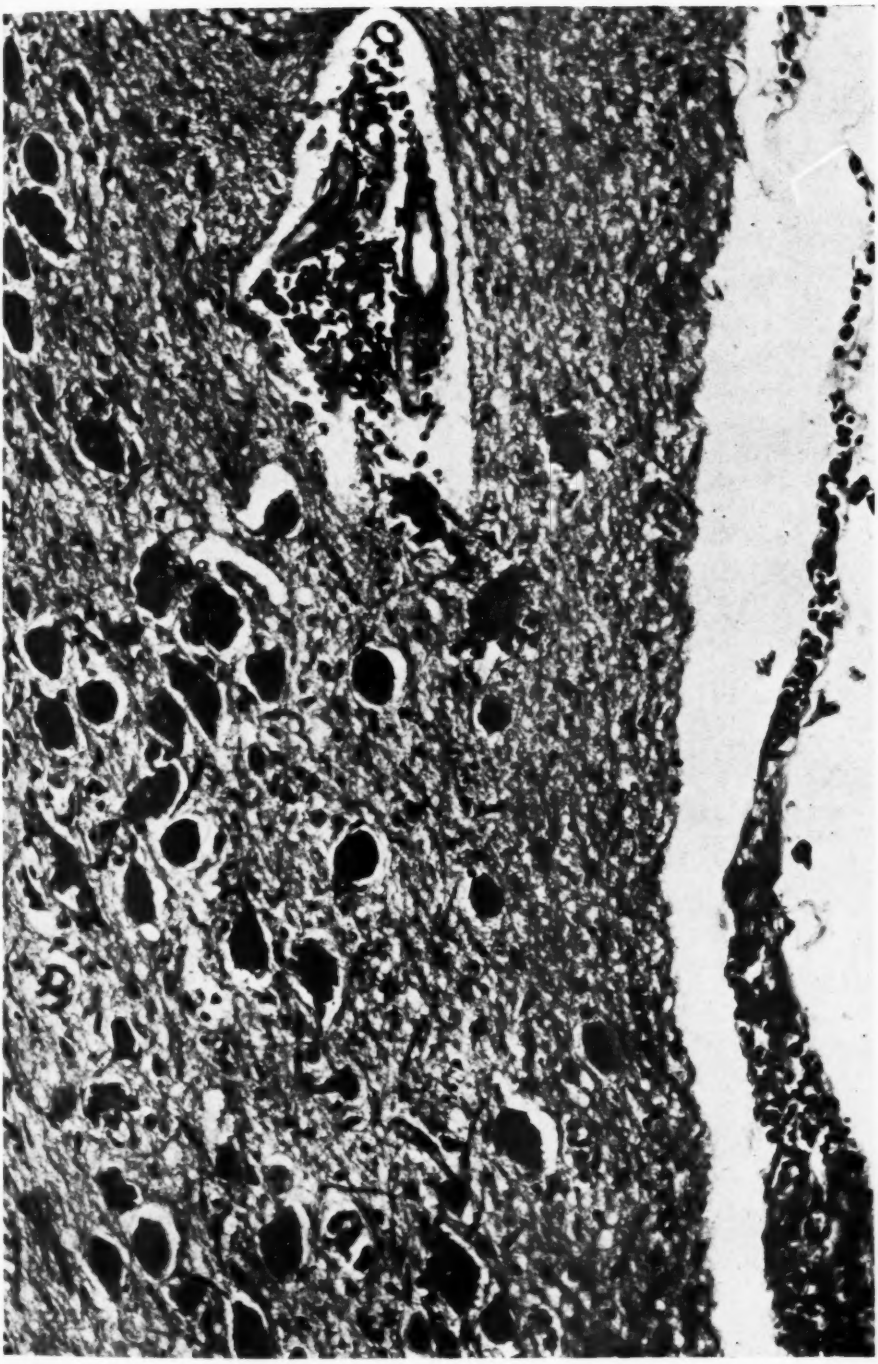
FIG. 75. Case 124. Brain. Mild degree of lymphocytic infiltration in leptomeninges; subpial edema. (See Fig. 73.) × 200.



Lucké Pathology of Fatal Epidemic Hepatitis

PLATE 120

FIG. 76. Case 76. Duration of hepatitis, 96 days. Brain; section through nucleus basalis lateral to anterior hypothalamic region. The meninges are infiltrated to a moderate degree with lymphocytes and histiocytes. In the subjacent cortex a similar infiltrate is noted around a small vessel. In nearly all ganglion cells the cytoplasm is swollen; in many the nucleus is shrunken and distorted. Around a few of the more disintegrated cells, satellitosis is evident. (See Figs. 67 to 69 for details of changes in ganglion cells.) × 240.



Lucké Pathology of Fatal Epidemic Hepatitis

THE STRUCTURE OF THE LIVER AFTER RECOVERY FROM EPIDEMIC HEPATITIS *

BALDUIN LUCKÉ, Lt. Col., M.C.

In the preceding paper,[1] the lesions produced by epidemic hepatitis were described as they occur in the liver and other organs in cases that terminated in death. The present paper is concerned with the far more numerous patients who recovered. What lesions, if any, remain in the liver, after the patient has been restored to health? Does the liver, when studied grossly and microscopically, show evidence of permanent damage, or, on the contrary, is repair complete, is lost tissue replaced, does healing occur without scarring? In a word, is clinical recovery accompanied by anatomic restitution so nearly complete as to justify the assertion that the liver has returned to its previous normal condition?

In order to answer these questions it was necessary to study patients who had recovered from a typical attack of epidemic hepatitis, and from whom later, as the result of accident or unrelated disease, the liver became available for examination. A series of such cases occurred during the outbreak of hepatitis in the Army in 1942. Of these, 14 cases have been studied. All these patients had typical hepatitis, the diagnosis being made by experienced clinicians during the course of an epidemic of the disease. Subsequent to the attack, 6 of the patients died from traumatic accident, and 6 from disease. In 2 other patients, a fragment of liver was removed during abdominal operations. These tissues were obtained from 1 to 14 months after clinical recovery from hepatitis.

To insure adequate sampling, blocks of liver were examined from a number of areas when possible. Sections from these were prepared by standard methods.[1] The pertinent clinical data and the results of the morphologic examination follow.

REPORTS OF CASES

Case 1

The patient was a white male, 27 years old.

Clinical Course. May 5, 1942: anorexia, weakness, constipation, headache, nausea, vomiting; later, jaundice. May 22: admitted; mildly jaundiced; liver not enlarged and not tender. May 24: appetite better; still complained of nausea; slight tender-

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ness over epigastrium. Improved steadily; by May 31 felt as well as before onset of illness, but trace of jaundice persisted; discharged from hospital. Temperature, pulse and respiration were normal throughout course.

Laboratory Findings. May 23: urine negative for albumin and sugar. Blood count: red blood cells, 5.0 million; white blood cells, 6,300; polymorphonuclear leukocytes, 41%; lymphocytes, 46%; monocytes, 9%; eosinophils, 1%; basophils, 1%. Icterus index, 75.

On June 15, 1942, this man was struck by an airplane and killed instantly. Duration of hepatitis, 26 days; interval between discharge and death, 16 days.

Post-Mortem Examination

Liver. Weight, 1810 gm. Surface was pale red and smooth; capsule, thin. Cut surfaces were homogeneous in appearance and showed no deviation from the normal.

Microscopically, the lobular pattern of the liver was accentuated because of infiltration of the periportal stroma with moderate numbers of polymorphonuclear leukocytes and lymphocytes. The reaction extended for some distance along the peripheries of some, but not of all, lobules. With connective tissue stains no increase in collagen formation could be demonstrated. The lobular architecture was everywhere normal. The hepatic columns were well oriented; the efferent veins occupied normal positions. Many efferent veins had somewhat swollen walls, but no increase in fibrous components was demonstrable. The bile canaliculi were inconspicuous. In the innermost region of some lobules a few liver cells were still lacking. The loss of parenchyma was minimal, probably much less than 1 per cent. Many cells, particularly in the central parts of the lobules, were binucleated, which indicates regenerative activity. The Kupffer cells in the central parts of many lobules contained yellowish pigment, probably lipofuscin.

This liver appears to be well on the way to complete restoration.

Case 2

This patient was a white male, 33 years old.

Clinical Course. About May 18, 1942: malaise, loss of appetite, nausea, vague abdominal pains, "dark" urine. May 16: admitted; skin and sclerae jaundiced; liver was 2 fingersbreadth below costal margin and slightly tender; icterus index, 75; white blood cells, 8,200. Progressed well during first 10 days in hospital. Hepatitis became quiescent, as was indicated by clearing of jaundice and return of index to within normal range. Course was afebrile throughout. On 10th day of hospital stay, temperature rose to 101° F. June 8: chill. June 11: a rough presystolic murmur; the condition was now regarded as subacute bacterial endocarditis, probably precipitated by the attack of catarrhal jaundice. June 27: died (50 days after onset of symptoms of hepatitis).

Post-Mortem Examination

Liver. Slightly smaller than usual. Cut surface showed no gross changes.

Microscopically, the lobular pattern was uniform throughout the organ. The periportal stroma, however, was infiltrated to a moderate degree with lymphocytes, polymorphonuclear leukocytes, and occasional pigmented histiocytes; no increase in collagen had taken place. The small biliary ducts were somewhat conspicuous. The hepatic cords converged in normal manner toward centrally placed, thin-walled veins. In general, the cells were well preserved and of uniform size. Around the central lobular vein the cellular arrangement was somewhat less uniform, and the size of the cells varied; here, large cells with prominent hyperchromatic nuclei, or with two nuclei, were common. An occasional bile "thrombus" was encountered in this region (Fig. 3).

Case 3

The patient was a white male, 30 years old.

Clinical Course. During April, 1942, had an attack of epidemic hepatitis. No clinical details are available. Presumably made a good recovery and returned to duty. On June 2, 1942, died from an unrelated disease: dementia praecox with marked cachexia and hypostasis of the lungs. Interval between discharge from hospital and death, approximately 3 months.

Post-Mortem Examination

Liver. Normal in size and shape; surface was smooth; cut surfaces were grossly normal.

Microscopically, organ was normal. There was no scarring. All traces of previous injury had disappeared. This liver may be considered as an example of the complete repair which in all probability occurs in the vast majority of cases of hepatitis.

Case 4

This patient was a white male, age unknown.

Clinical Course. June 23, 1942: anorexia. July 3: jaundice. July 7: admitted. Steadily improved under treatment and was discharged on July 10. August 12: died of gunshot wound. Duration of hepatitis, approximately 17 days; interval between discharge and death, 32 days.

Post-Mortem Examination

Liver. Weight, 1440 gm. Surface was smooth. Cut surfaces revealed no gross changes.

Microscopically, the organ showed evidence of extensive regeneration. The lobular architecture was preserved; most lobules had a normal structural pattern, *i.e.*, cords of hepatic cells converged toward a common efferent vein. The argentophilic reticulum was intact, and its fibrils were normally delicate. Here and there, however, considerable variation in the size and shape of the lobules was encountered; many were uniformly enlarged, while others showed an eccentric in-

crease in size, so that the shape was distorted. In all lobules the great majority of the cells had stored an abundance of glycogen, and their nuclei were normal in appearance. Proliferative changes were most evident in the neighborhood of the central veins and at the lobular peripheries, where many cells were large and had smooth cytoplasm without glycogen granules and with prominent hyperplastic nuclei; binucleated and multinucleated forms were commonly encountered.

Bile canaliculi were inconspicuous, and retention of bile within cells or within hepatic columns was not evident.

The periportal canals were normal. In most areas their stroma was normal in quantity and quality; here and there, however, it was somewhat more abundant, extended for a short distance along the lobular peripheries and was infiltrated with moderate numbers of histiocytes, lymphocytes and polymorphonuclear leukocytes. In such regions the small biliary ducts usually had undergone slight proliferative changes; that is to say, their number appeared to have doubled or tripled.

Occasionally, small clumps of histiocytes laden with lipofuscin were encountered within the periportal stroma or in the central zones of lobules. The pigment probably was derived from liver cells and served as an indicator of a disintegrative process long since passed.

In summary, the structural restitution of the liver was nearly complete. Traces of inflammatory reaction still lingered in the periportal stroma of some lobules. The hepatic cells were storing glycogen in a normal manner; there was no evidence of interference with the vascular supply or the biliary drainage.

Case 5

This patient was a white male, 23 years old.

Clinical Course. May 20, 1942: anorexia, headache, "dark" urine, constipation, later, jaundice. May 24: admitted; blood pressure, 124/80. Course in hospital was uneventful. July 3: discharged and returned to duty.

Laboratory Findings. May 24 and June 16: urine negative for albumin; positive for bile. July 3: urine negative for albumin and bile. May 24: red blood cells, 4.5 million; white blood cells, 7,700; polymorphonuclear leukocytes, 64%; lymphocytes, 36%. Icterus index: June 8, 30; June 16, 15.

July 20: Died as result of bullet wound. Duration of hepatitis, 43 days; interval between discharge and death, 17 days.

Post-Mortem Examination

Liver. Size and color were normal. Capsule was smooth. Cut surface showed normal architecture.

Microscopically, the liver showed well advanced repair. There was

complete preservation of the lobular pattern. The portal canals and their stroma were normal; no proliferation of smaller biliary ducts was evident. The hepatic columns radiated in normal manner toward the central efferent veins; the strands of supporting reticulum were unbroken. The appearance of the central parts of the lobules contrasted with that of the peripheral zones: many of the central cells were larger and had richly chromatic nuclei; binucleated cells were commonly encountered. These central cells obviously were newly formed by multiplication. In contrast, the peripheral cells were smaller, their nuclei less prominent, and their cytoplasm was laden with glycogen. In some lobules the central cells were still loosely arranged and the tissue contained a few scattered lymphocytes as indicators of past damage. Many of the efferent veins still had a swollen edematous wall, but endophlebitis, such as is commonly observed in cases terminating fatally, was not present.

The conspicuous feature in the central lobular regions was the number of large pigmented macrophages, probably mobilized Kupffer cells; they had ingested a dark brown granular pigment which gave no reaction with iron stains but did react with fat stains.

Repair of the liver may be considered practically complete (Fig. 4).

Case 6

The patient was a white male, age unknown.

Clinical Course. Early in July, 1942, complained of nausea and vomiting and later became jaundiced. July 12: admitted. Course uneventful. July 25: discharged and returned to duty.

September 9: killed in automobile accident. Course of hepatitis, approximately 25 days; interval between discharge and death, 45 days.

Post-Mortem Examination

Liver. Weight, 1795 gm. Surface was smooth. Cut surface showed no significant alteration.

Microscopically, the organ showed little evidence of previous damage aside from the large size and irregular shape of many lobules. The portal canals were normal and their supporting stroma was of average quantity and normally cellular except in a few areas where slight proliferation had led to the extension of thin stromal strands part way about adjacent lobules. Practically all lobules had a normal structural pattern. The cells everywhere were well preserved; in the central lobular zones there were occasional clumps of histiocytes containing lipofuscin; here, binucleated liver cells were common. Many efferent veins had slightly thickened walls. Restoration of this liver is practically complete (Fig. 7).

Case 7

This patient was a white male, 25 years old.

Clinical Course. During August, 1942, had an attack of acute catarrhal jaundice. No clinical details are available. October 18: was operated upon for acute appendicitis. October 22: suddenly went into shock and died as result of obstruction of right pulmonary artery by embolus, probably arising from thrombus in left hypogastric vein. Duration of hepatitis unknown; interval between discharge and death, approximately 2 months.

Post-Mortem Examination

Liver. Weight was approximately 1300 gm., and consistency was normal. Surface was smooth. Capsule was thin. Cut surfaces had normal color and showed normal architectural pattern. No evidence of pathologic change was found.

Microscopically, the architecture was normal. The portal canals and their stroma were completely normal. The hepatic lobules were of average size, and their cords converged in normal manner toward the centrally placed efferent veins. The individual liver cells were normal in size, shape and details of structure. The canaliculi were inconspicuous. No bile "thrombi" were evident. The efferent veins had normal walls. The sinusoids were well filled, and their lining was intact. Reticulum stains showed that the framework had a normal pattern. No traces of previous injury were observed in this liver; restoration following the attack of epidemic hepatitis was complete (Figs. 5 and 6).

Case 8

The patient was a white male, 31 years old.

Clinical Course. About June 13, 1942: loss of appetite, nausea, vomiting, headache, weakness, malaise, "dark" urine. June 19: admitted; sclerae and skin icteric; no tenderness over abdomen; liver and spleen not palpable. Stay in hospital uneventful. June 26: free of symptoms; felt well; jaundice disappearing. June 28: discharged in good condition though still slightly jaundiced.

Went on drinking bout and died on July 5 from acute alcoholism. The alcohol which was drunk was probably impure and unfit for consumption. Duration of hepatitis, approximately 15 days; interval between discharge and death, 7 days.

Post-Mortem Examination

Liver. Except for slight congestion, grossly normal.

Microscopically, the liver was involved in a uniform manner. The lobules presented a similar appearance; in each the columns of liver cells were normally arranged and converged toward a central vein. But the cells immediately surrounding the vein had been destroyed, so that the innermost part of each lobule appeared empty. At the central terminals of the columns the cells were large and had prominent hyperchromatic nuclei; many were binucleated; some had become detached. The cells here were obviously multiplying and restoring lost tissue.

Regenerative activity, however, was not confined to the lobular centers. At the periphery of each lobule many minute biliary ducts were seen at the terminals of the hepatic columns, and larger biliary ducts with numerous branches were present in the periportal stroma. The liver cells also were multiplying, as was indicated by the presence of many richly chromatic nuclei and binucleated cells. This liver exemplifies the fact that the peripheral zone next to the portal area usually takes an active part in the restoration of injured tissue.

Apart from the loss of cells and the multiplication of neighboring survivors, there were no noteworthy changes in the hepatic cords. The component cells had a somewhat lumpy cytoplasm, probably as a result of glycogen storage; no fat could be detected by appropriate stains. The nuclei were normal. The bile canaliculi were inconspicuous and contained no inspissated material.

The supporting reticulum was completely preserved, and where liver cells had been lost unbroken lines of reticulum swept toward the central vein. The latter, in most lobules, had a normal wall; occasional veins with somewhat thickened walls were encountered, but endophlebitis was nowhere seen. The sinusoids were patent and filled with blood.

An indicator of previous destruction of liver cells was furnished by the conspicuous number of swollen and pigmented Kupffer cells. Many had become detached and isolated or, in small clusters, lay in the stroma, particularly in the "empty" center of the lobules and in the periportal stroma. The pigment stained the cells diffusely; it occurred as small brown granules, gave no reaction for free iron, did not have the appearance of bile and stained deeply with Sudan and with silver. The latter, probably by encrustation, caused the granules to appear much coarser than they seemed unstained. This pigment was probably derived from the breakdown of liver cells; it belongs in the large and heterogeneous group of lipochromes.

The peripheries of the lobules were clearly marked, and here the portal stroma still contained a moderate excess of lymphocytes and plasma cells, occasional leukocytes and many pigmented histiocytes. The proliferative changes in the bile ducts, mentioned previously, helped in making the lobular peripheries prominent. The portal canals were normal. There was no evidence of connective tissue overgrowth. It is to be expected that the reactive changes will eventually subside.

Summarizing, this liver shows evidence of destruction of cells in the innermost portions of the lobules (Fig. 2). Through regenerative multiplication of the adjacent surviving cells all but approximately 5 per cent of the parenchyma has been restored. The small amount still lacking to complete restoration is negligible, in view of the enormous

reserve of hepatic tissue. What part alcohol and associated faulty diet played in the hepatic picture of this case is problematic. From the history it is evident that death was due directly or indirectly to excessive use of alcohol; moreover, the alcohol used was probably impure and unfit for consumption. There is a distinct possibility that because of excessive consumption of alcohol complete restoration of hepatic tissue has been delayed.

Case 9

The patient was a white male, 23 years old.

Clinical Course. May 22: anorexia, nausea, fatigue, constipation; later, "dark" urine. May 28: icteric sclerae. June 4: admitted; moderately jaundiced; slight tenderness in right upper quadrant; liver not palpable. The course was afebrile. Appetite remained poor for several days, then gradually returned to normal.

Laboratory Findings. Icterus index: June 8, 90; June 15, 50; June 17, 30. June 4: urine was positive for bile. June 5: red blood cells, 4.9 million; white blood cells, 4,700; polymorphonuclear leukocytes, 72%; lymphocytes, 28%.

October 29: killed in an automobile accident. Duration of hepatitis, 25 days; interval between discharge and death, 102 days.

Post-Mortem Examination

Liver. Grossly normal except for a traumatic tear through the capsule.

Microscopically, the architecture of the liver was preserved. The portal canals were normal. The cellular contents of the supporting stroma were within the normal range. The hepatic lobules had average size and shape; their cords converged toward centrally placed efferent veins. The latter usually had thin walls, though here and there a vein with a thick edematous wall was encountered. The individual liver cells were normal in staining qualities; occasional binucleated forms were present, particularly in the more central portions of the lobules. The reticulum framework was normal in arrangement and amount. This liver appeared to have been completely restored.

Case 10

This patient was a white male, 35 years old.

Clinical Course. About July 15 he was seen as an out-patient because of icteric sclerae, with the usual symptoms of catarrhal jaundice. Because of mild character of the attack, he was not placed in hospital. July 22: dyspnea and signs of bronchopneumonia developed. July 23: died.

This patient had an attack of mild epidemic hepatitis and a virulent form of hemorrhagic bronchopneumonia developed while he was recovering from the hepatitis. The total duration of the hepatitis is difficult to estimate from the history, but it was probably 2 weeks. No further information is available.

Post-Mortem Examination

Liver. Average size, had a smooth surface and thin capsule. Cut surface showed no significant deviation from the normal.

Microscopically, the architecture was preserved. The portal stroma was normally cellular; the bile ducts showed no proliferative changes. Small groups of liver cells had been lost in patches which were usually adjacent to the central lobular veins. In these regions the sinusoids were moderately engorged, and scattered histiocytes and lymphocytes were present. The efferent veins had somewhat swollen walls, some of which were sparsely invaded by lymphocytes. Active regeneration of liver cells in the destroyed areas was indicated by the presence of numerous binucleated and multinucleated cells. In many lobules the intracolumnar canaliculi were conspicuously dilated and contained clumps of inspissated bile.

This liver is an example of early restoration which is not as yet complete (Fig. 1).

Case 11

This patient was a white male, 35 years old.

Clinical Course. In June, 1942, became fatigued and was intermittently nauseated. During July, lost his appetite; tenderness developed in the upper abdomen; the urine became dark; and jaundice appeared, which gradually deepened. About August 1 the stools became clay-colored. Diagnosis of acute catarrhal jaundice was made on August 3. August 8: patient was transferred to a General Hospital; was nauseated and deeply jaundiced; complained of itching but had no other objective or subjective symptoms; blood pressure, 104/70. After 1 week in bed he improved considerably. September 2: felt much better; stools darker; liver slightly enlarged and tender. September 6: temperature, 100° F.; complained of discomfort in upper abdomen. September 14: no complaints; jaundice much improved; edge of liver no longer below costal margin. October 2: in good condition. From then on progress was uneventful. November 11: patient discharged and returned to duty.

On December 13, signs of perforated peptic ulcer developed. At operation the perforated area was found on the anterior wall. The liver had a normal appearance. The edge was slightly thickened. A specimen approximately 2 by 1.5 cm. was removed for examination. Duration of hepatitis, approximately 6 months; interval between discharge and taking of specimen for biopsy, 32 days.

Laboratory Findings.

Date	Icterus index	Date	Icterus index	Date	Icterus index
August 10	121	Sept. 6	43	Oct. 7	15
August 17	200	Sept. 12	31	Oct. 23	12
August 24	135	Sept. 21	26	Nov. 6	16
August 30	100	Sept. 30	23	Nov. 11	12

Blood Count. August 14: red blood cells, 4.2 million; white blood cells, 8,500; polymorphonuclear leukocytes, 59%; lymphocytes, 37%. October 30: red blood cells, 4.3 million; white blood cells, 8,800; polymorphonuclear leukocytes, 69%; lymphocytes, 31%.

Microscopic Examination of Specimen Removed from Liver

Microscopically, the specimen consisted of a triangular piece of tissue measuring 17 mm. at the base and 8 mm. in height. The capsule was of average thickness. Its vessels were engorged and a few capil-

laries had ruptured; the resulting small hemorrhages were probably due to the operative procedures. The architectural pattern of the liver was normal. The size and shape of the lobules were unaltered; the lobules were composed of normal hepatic cords which converged toward central efferent veins. The individual cells were of average size and their nuclei had a normal appearance; the cytoplasm was dotted with many small irregular vacuoles which indicated normal glycogen storage. Occasional cells contained a few sharply contoured droplets of fat. The reticular framework of the lobules was intact and was normally delicate. The efferent hepatic veins and the portal canals were likewise normal. The portal stroma was infiltrated by moderate numbers of lymphocytes and leukocytes. In some of the portal vessels, and here and there in the sinusoids, were small clusters of well preserved leukocytes which probably indicated a recent infection or destructive process in the abdomen. Hepatic damage had been successfully repaired, and restoration may be considered complete (Fig. 8).

Case 12

The patient was a white male, 25 years old.

Clinical Course. June 16, 1942: admitted to hospital with history of feeling tired and having poor appetite for past 2 weeks. Sclerae became jaundiced on June 15. On admission he was ambulatory and not acutely ill. Liver was not tender or palpable. Clinical course was uneventful and afebrile. Remained in hospital until December 15 and was then given sick leave. In January, 1943, upper abdominal pain developed which was not affected by food, activity, or pressure; he was not nauseated and did not vomit. February 13, 1943: x-ray examination revealed numerous stones in the gallbladder. Impression of the surgeons was that the stones originated during attack of acute hepatitis in summer of 1942. March 23: cholecystectomy showed the gallbladder had slightly thickened walls and contained seven dark brown stones. At this operation a small portion of the liver was removed for examination.

Duration of hepatitis, approximately 3 months; interval between discharge and taking of specimen for biopsy, approximately 4 months.

Laboratory Findings.

Date	Icterus index	Date	Icterus index	Date	Icterus index
July 7	230	August 1	32	Jan. 1	17
July 10	125	August 11	26	Jan. 8	18
July 19	41	Sept. 6	25	Jan. 11	18
July 24	49	Sept. 13	12		

Microscopic Examination of Specimen Removed from Liver

Two fragments of tissue, each measuring approximately 18 by 5 mm. were examined. Both were from the region of the gallbladder. The tissue changes in the two sections were somewhat dissimilar. In one, the architectural pattern was normal, although several lobules were exceptionally large. The portal canals and their supporting stroma

showed no alteration. The efferent veins had thin walls; occasional ones were eccentrically located. The liver cells generally were large and of uniform size; all were well preserved; their nuclei were intact and their cytoplasm contained a normal store of glycogen. In the other section thin strands of stroma partly encircled some of the lobules. Otherwise the appearances of the two sections were similar. The slight focal perilobular fibrosis was similar to that which commonly occurs in regions of the liver adjacent to a chronically inflamed gallbladder; it probably represented an extension of a local chronic inflammatory reaction. Restoration of the hepatic parenchyma was complete.

Case 13

The patient was a white male, 32 years old.

Clinical Course. About June 27, 1942, generalized aching, malaise, anorexia and nausea; later, jaundice and "dark" urine. July 3: admitted; temperature, pulse and respiration were normal; liver not definitely palpable and not tender. While in hospital jaundice deepened markedly. Patient remained afebrile except for occasional rises in temperature up to 99.2° F. September 1: discharged and given 30 days sick leave.

Laboratory Findings. On admission, icterus index was 25; it gradually increased during the next 3 weeks to a maximum of 230, after which it dropped; it was 10 at the time of discharge. During the early stages of jaundice, the urine contained bile. Blood chlorides on July 28 were 412 mg.%.

April 10, 1943: died from bullet wound. Duration of hepatitis, approximately 3 months; interval between discharge and death, 8 months.

Post-Mortem Examination

Liver. Weight, 1580 gm. External surface was smooth, capsule was thin, and cut surfaces were grossly normal.

Microscopically, the lobular pattern was completely normal. The size of the lobules in general varied but little from the average; in occasional areas a few lobules appeared enlarged, and a few others were reduced in size. The portal triads also were normal; most of them lay in a scanty stroma which here and there contained a slightly increased number of lymphocytes. The hepatic cords converged in regular manner toward thin-walled, centrally placed veins. The liver cells were fairly uniform in size; their nuclei were well preserved; their cytoplasm had an abundant store of glycogen. Only one indicator of previous damage remained: in some lobules the cells immediately adjacent to the central veins were large and had prominent, deeply chromatic nuclei; binucleated cells were commonly encountered.

Case 14

This patient was a white male, 22 years old.

Clinical Course. July 7, 1942: loss of appetite, general malaise, nausea, vomiting, "dark" urine; later, jaundice. Uneventful course in hospital. July 24: discharged.

Patient was readmitted about 1 year later with evidence of chronic glomerulonephritis and died on September 24, 1943. Diagnosis was confirmed by post-mortem examination. Duration of hepatitis, approximately 17 days; interval between discharge and death, 14 months.

Post-Mortem Examination

Liver. Grossly normal.

Microscopically, the lobules were normal in size, shape and arrangement. The periportal stroma was scanty and contained no excessive number of cells. The bile ducts and vessels were normal. The hepatic cords had the usual radiating pattern, and their component cells were normal. The efferent veins had thin walls. Repair of this liver was complete.

Comments on the Gross and Microscopic Appearance of the Liver

Without exception, the liver in each of the 14 cases studied had macroscopically a normal appearance. The size and shape were within normal limits, the surface was smooth, the capsule thin, the consistency unaltered. The structural pattern of the cut surface was uniform.

Microscopically, the appearance varied somewhat, depending on the length of time between the attack of hepatitis and death or biopsy. In all cases, however, the lobular architecture was preserved, and the reticulum frame was intact.

In summarizing the microscopic appearances it is convenient to describe first the hepatic parenchyma, and afterwards the periportal stroma, ducts, and vessels.

The Hepatic Parenchyma

In 2 cases, restitution of lost parenchyma was not yet complete. One of these patients (case 10) had died of intercurrent infection during convalescence from hepatitis, the other (case 8) from acute alcoholism only 1 week after leaving the hospital. In these livers the process of repair could still be observed.

Thus in case 10, in the central portions of most lobules, the columns of liver cells appeared broken, and small patches of parenchyma were lacking (Fig. 1). Many bile canaliculi were dilated with clumps of bile. Numerous binucleated or multinucleated liver cells indicated active regeneration.

With the exception of these two examples of incompleted repair, restitution of hepatic parenchyma was very nearly complete in 3 cases, and entirely complete in the other 9. The patients (cases 1, 2 and 5) of the first group died within approximately 3 weeks after clinical re-

covery from jaundice. The lobular structure in all had been restored completely, and only an occasional liver cell was still lacking. Some traces of previous damage, however, were discernible. Thus, in case 2 numerous large cells with one or two hyperchromatic nuclei were clustered in the immediate neighborhood of the central lobular vein, and a few canaliculi in this region still contained bile "thrombi" (Fig. 3). The appearance of the reticulum in the central zone of a lobule, *i.e.*, in the previously damaged region, is shown in another, similar case (Fig. 4). The arrangement, delicacy and complexity of the reticulum network are entirely normal.

We now come to the group of 9 cases in which the lost tissue was entirely restored. In these cases a longer time, from 1 to 14 months, had elapsed since discharge from the hospital. In most of them the structure of the lobules was normal, while in 2 cases slight variations in size and shape of some of the restored lobules suggested previous injury and subsequent repair. The hepatic cells had a healthy appearance. Fat and glycogen storage appeared to be within the usual limits, and the cytoplasm contained no abnormal constituent. In summary, restitution of lobular parenchyma was very nearly complete 3 weeks after recovery from hepatitis, and entirely complete 1 month after clinical recovery.

The Periportal Stroma

A slight to moderate increase in cellularity was observed in nearly one-half the cases. The cells were mainly lymphocytes and larger mononuclear cells, but polymorphonuclear leukocytes were also present, although in relatively small numbers. This increase in cellularity was generally confined to the livers of those patients who had recovered less than a month previously.

In 3 cases of the series (1, 6, and 12) thin strands of cellular connective tissue were found to extend for some distance along the periphery of the lobules. Encircling of lobules or significant scarring was observed in none. Moreover, the slight fibrosis was focal and never diffuse.

Biliary Ducts

In 3 cases the small bile ducts showed a slight to moderate degree of proliferation. Conspicuous branching, such as is a prominent feature in fatal cases, occurred in none.

Vasculature

The branches of the portal vein and of the hepatic artery were normal. The sinusoids had an average blood content. The central lobular vein in 4 cases had somewhat swollen walls; in one instance

(case 10) the subendothelial intima was sparsely infiltrated with lymphocytes (this patient died during early convalescence). Definite endophlebitis, a notable feature in fatal cases, was not observed. None of the cases showed perivenous condensation of reticulum or of collagenous connective tissue.

Histiocytes with Lipofuscin Granules

In 5 cases pigmented histiocytes, isolated or in small clusters, were found, particularly in the central zones. These cells may be regarded as indicators of past destruction of liver cells.[1]

Summary of Microscopic Findings

The results of microscopic examinations may now be summarized as follows. In all 14 cases of the series, the lobular architecture was preserved, and the reticular framework was intact. Two patients who had died during, rather than after, convalescence showed incompleted repair, in the sense that small patches of hepatic parenchyma were still lacking in the central zone of the lobules.

In the remaining 12, restitution of the parenchyma was very nearly complete in 3, and entirely so in 9 cases. In the periportal stroma traces of inflammatory reaction lingered in 6 cases; in 3 of them slight focal increase of the stroma had taken place. Scarring was present in none. Slight proliferation of the small bile ducts was observed in 3 cases. Phagocytic cells containing lipofuscin were found in 5 cases. In 4 cases the walls of the central lobular veins were somewhat thickened. Briefly, the changes observed represent slight traces of preceding hepatic injury; they do not indicate persistent or progressive damage. The hepatic parenchyma has been restored in normal manner.

Discussion

The liver is known to have great powers of regeneration.[2-4] Whether after destruction of liver substance regeneration is complete, with restoration to the previous normal condition, or whether extensive scarring and permanent damage occurs, depends on two circumstances.[5-8] The first is whether the damage is confined to the cells of the hepatic parenchyma. If so, regeneration may be complete, as these cells have great ability to multiply and restore themselves. If, however, damage involves the reticulum of the lobule or if the hepatic veins or the portal vessels and their stroma are destroyed, then restoration of the hepatic lobule occurs in an abnormal fashion, with more or less extensive fibrosis, and permanent damage.

The second factor is whether damage to the liver occurs only once

or whether insults are repeated. Recovery from a single injury affecting only the parenchymal cells may be complete whereas repeated destruction of cells, especially at short intervals, may result in fibrosis and permanent damage.

Such is the pattern of reaction of the liver to injury, as ascertained both through experimental work and from study of human material.

Thus Whipple and Sperry[9] found that after chloroform had been administered to dogs in amounts sufficient to cause necrosis of two-fifths or three-fifths of every hepatic lobule, the livers of surviving animals regenerated perfectly in 3 weeks. Similar complete recovery of the liver occurred after single doses of carbon tetrachloride (Cameron and Karunaratne[8]), though not after repeated doses unless time for regeneration was allowed between doses.

In monkeys infected with yellow fever (Klotz and Belt[6]), two-thirds died and showed extensive liver necrosis, but of those that recovered all showed complete and scarless hepatic regeneration.

That the liver of epidemic hepatitis conforms to this reaction pattern has been shown by the present study. In hepatitis the destruction of liver cells occurs acutely, not as a continued process with progressive damage and fibrosis. Further, destruction is confined to the hepatic cells; reticulum, intralobular vasculature, portal canals and stroma are left intact. Therefore, complete regeneration is possible. The results of this investigation show that complete regeneration is in fact the rule; that in nonfatal cases of hepatitis there is no progressive or residual damage to the liver; that just as clinical recovery is complete, so the liver parenchyma is restored to its previous anatomic condition.

There have recently appeared two important papers[7,10] on the pathology of the liver in epidemic hepatitis. In a number of patients, specimens for biopsy were taken both during the acute attack and after recovery. At the first biopsy there were many necrotic liver cells in the center of the lobules, but at the second biopsy the liver was found restored to normal, as is shown by photographs. The authors concluded that most cases of hepatitis end in complete recovery of the liver. In a few other cases, scars were found. The histories of these patients suggest that the scarring antedated hepatitis or else that the patients suffered from liver diseases other than hepatitis. Furthermore, a sharp distinction must be made between harmless focal scars and cirrhosis, a progressive disease affecting the liver diffusely. This distinction has lately been re-emphasized in an excellent paper by Karsner.[11]

Lastly, there is a wealth of clinical evidence which favors the view that acute hepatitis, either in its epidemic or its sporadic form, tends to heal without leaving a permanently damaged liver such as is found

in cirrhosis. There has been no increase in cirrhosis following the numerous outbreaks of hepatitis. In fact, Rössle [12] stated that during the first World War and in the years immediately following, 1914 to 1922, the incidence of cirrhosis noticeably decreased, whereas during the same period occurred a rise in the incidence of so-called catarrhal jaundice.

It must not be forgotten that both epidemic hepatitis and cirrhosis of the liver are common diseases; hence patients suffering from cirrhosis may sometimes give a history of a previous attack of "catarrhal jaundice." Such a history does not, however, prove a causal relationship.

Summary

Only a small fraction of the cases of epidemic hepatitis terminate in death. The great majority of patients make a clinical recovery that is complete and apparently permanent. Whether in these recovered cases the liver is fully restored to a normal condition, or whether, on the contrary, there is residual damage or even progressive pathologic changes is the subject of this investigation.

The structure of the liver was studied in 14 cases after recovery from epidemic hepatitis. From 1 week to 14 months after the attack, these livers became available for examination as the result of fatal accident or unrelated disease.

Grossly all livers appeared entirely normal. Microscopically the appearance varied somewhat with the elapsed time since recovery, but in every instance the integrity of all liver lobules was preserved. In 2 patients who died of intercurrent disease during convalescence, repair of the liver lobules was still under way, and cells lost from the central part of the lobules had not been entirely replaced. In 3 cases examined within a month after clinical recovery, the liver lobules were entirely reconstituted, but slight evidence of previous damage still remained. In 9 other cases examined from 1 to 14 months after recovery, the liver parenchyma was restored completely. In less than half of the cases, traces of previous damage were found in the portal triads, but in none was found significant scarring.

It is concluded that complete restoration of hepatic parenchyma occurs in nonfatal cases of hepatitis. This conclusion is in agreement with what is known about the ability of the liver to regenerate. Regeneration is usually complete providing that destruction is acute and injury not continued, and providing that destructive changes involve only the hepatic cells, not the framework or vessels. This is the case in epidemic hepatitis. In the present investigation there was found no evidence of permanent damage to hepatic parenchyma, and restoration of the liver was practically complete.

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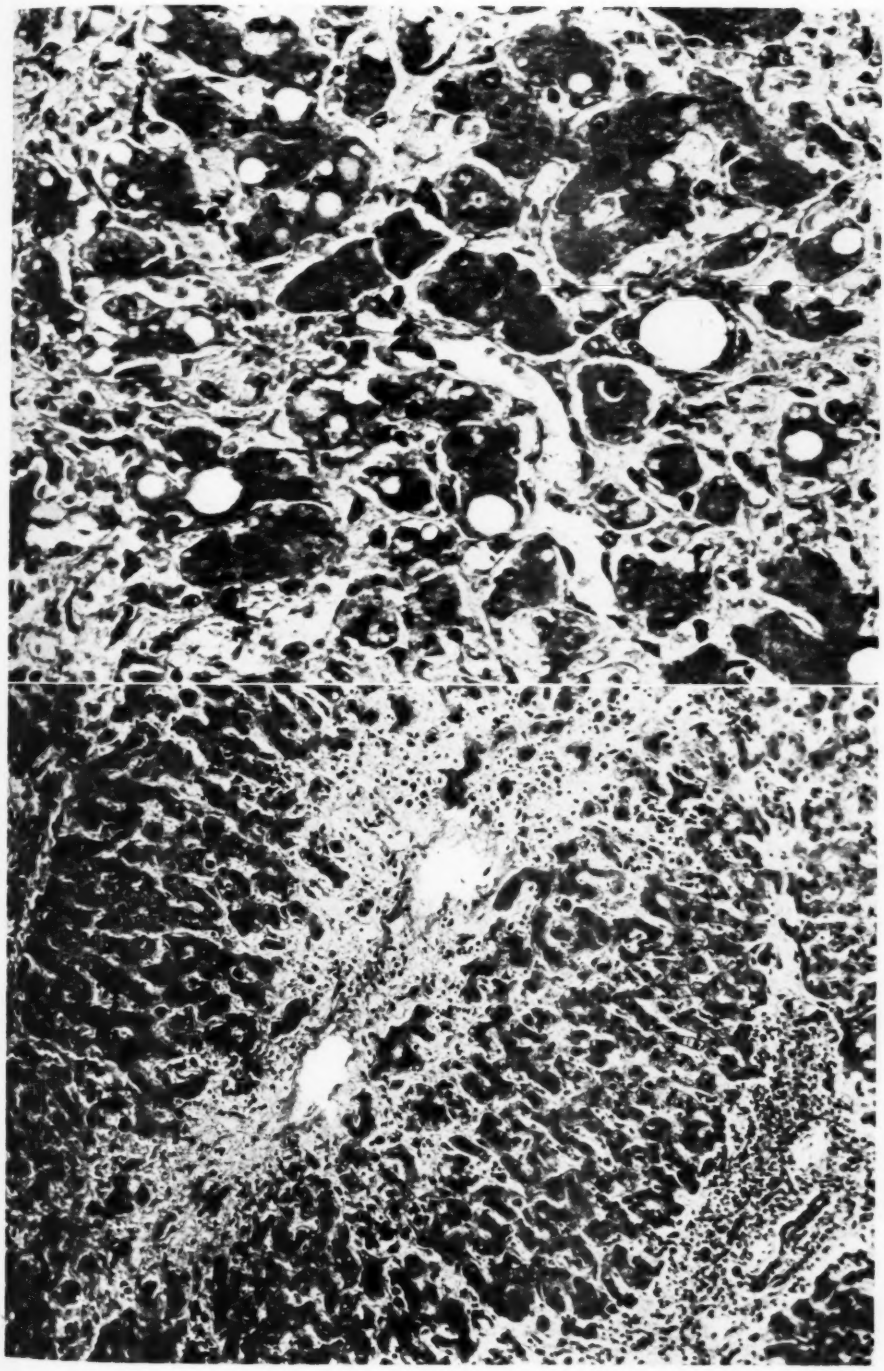
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 121

FIG. 1. Case 10. Area from central part of a lobule in which restoration of parenchyma is incomplete; small groups of liver cells are lacking. The sinusoids are distended. The stroma is edematous, and through it are sparsely scattered lymphocytes and histiocytes. In two hepatic columns, shown in cross section, the canaliculi are dilated with bile. × 500.

FIG. 2. Case 8. In the central part of this lobule the destroyed parenchyma has not as yet been completely restored. The hepatic cords converge in normal manner toward the lobular center. At the terminals of the cords the cells are large, and have prominent hyperchromatic nuclei; these regenerating cells are invading the empty stroma. The latter contains a moderate number of lymphocytes and histiocytes. The periportal stroma shows similar cells, and a few small proliferating bile ducts. There is no evidence of new formation of collagenous connective tissue. × 100.



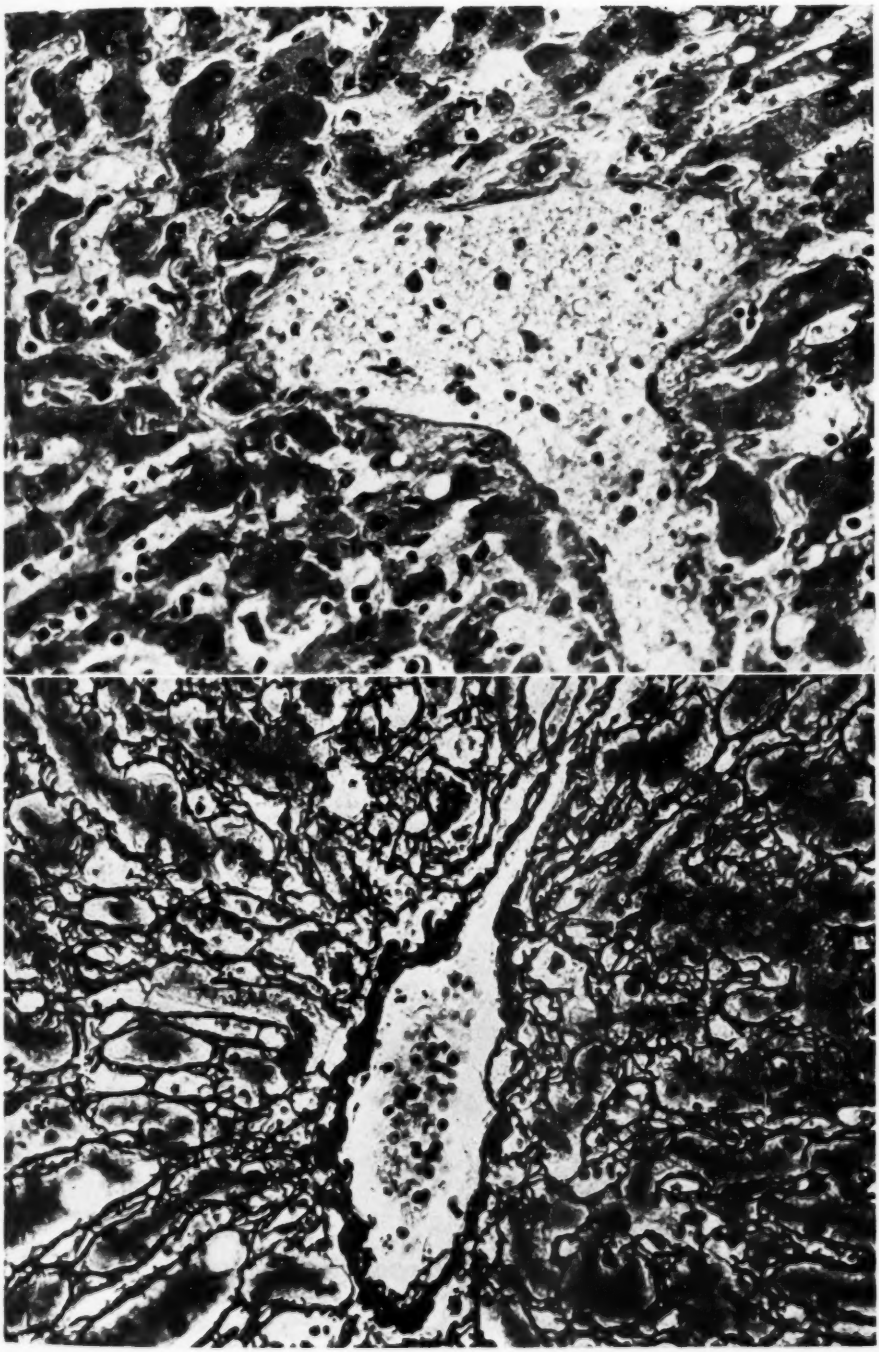
Lucké

Recovery from Epidemic Hepatitis

PLATE 122

FIG. 3. Case 2. Central part of a lobule showing an efferent vein and the adjacent hepatic parenchyma. The wall of the vein is normal. The parenchyma has been restored; a number of liver cells are conspicuously large and have one or two prominent hyperchromatic nuclei. In one of the hepatic cords a bile "thrombus" may be seen above and to the left of a large vein. × 500.

FIG. 4. Case 5. Central part of a lobule. The reticulum has a normal pattern. Here and there, a few liver cells are still lacking. The stroma contains numerous macrophages with ingested lipofuscin, the granules of which are blackened by the silver stain. Elsewhere, the reticular meshes enfold normal regenerated liver cells. Wilder's reticulum stain. × 500.



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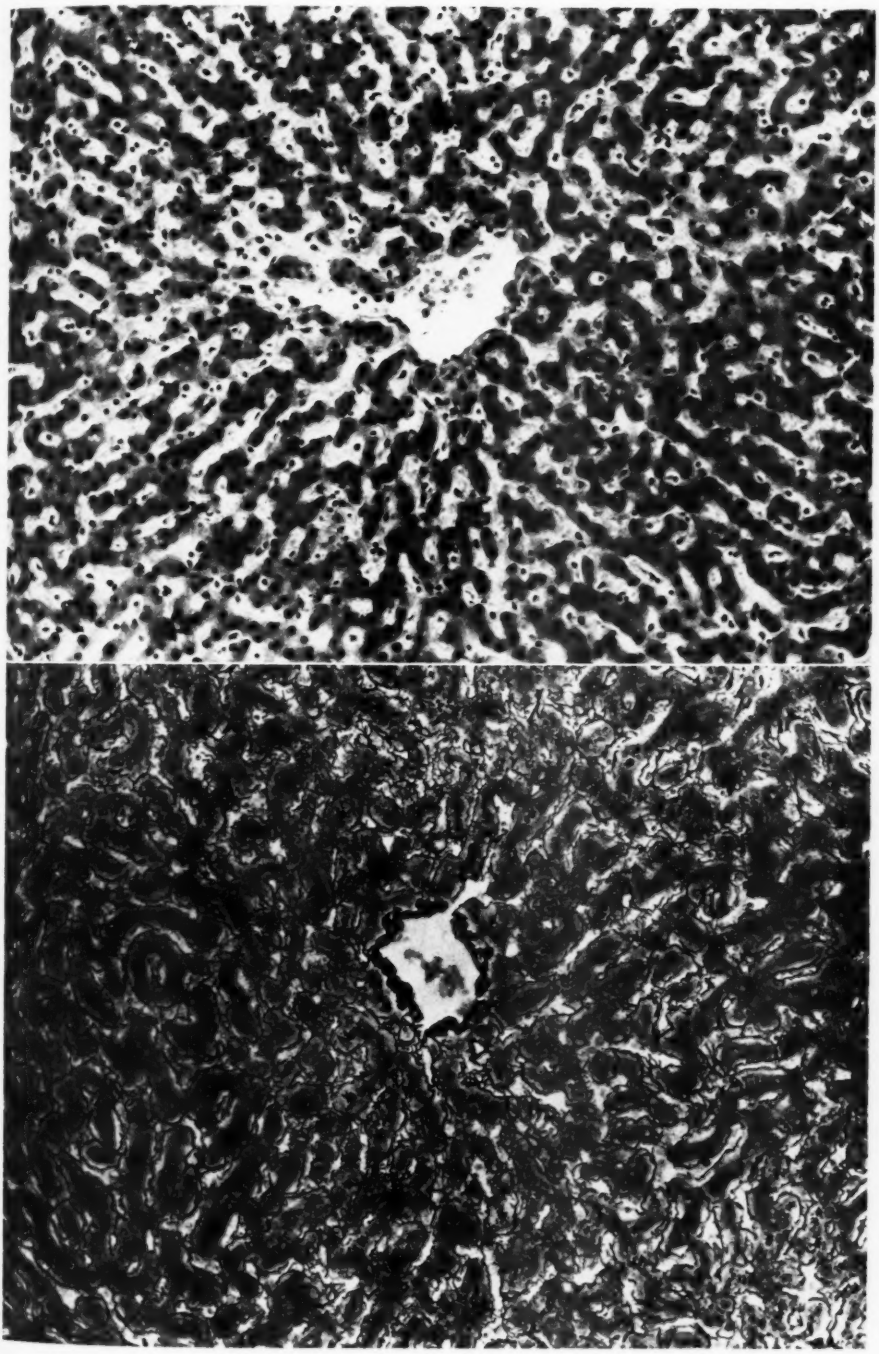
4

Lucké Recovery from Epidemic Hepatitis

PLATE 123

FIG. 5. Case 7. A hepatic lobule with columns of normal appearing hepatic cells converging toward a thin-walled central vein. No traces of previous injury can be observed. Restoration following the attack of epidemic hepatitis is complete. × 150.

FIG. 6. Case 7. Another lobule from the liver shown in preceding figure. The section has been stained by Wilder's method. The reticulum framework has a normal pattern of arrangement; its meshes enfold normal liver cells. × 150.



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6

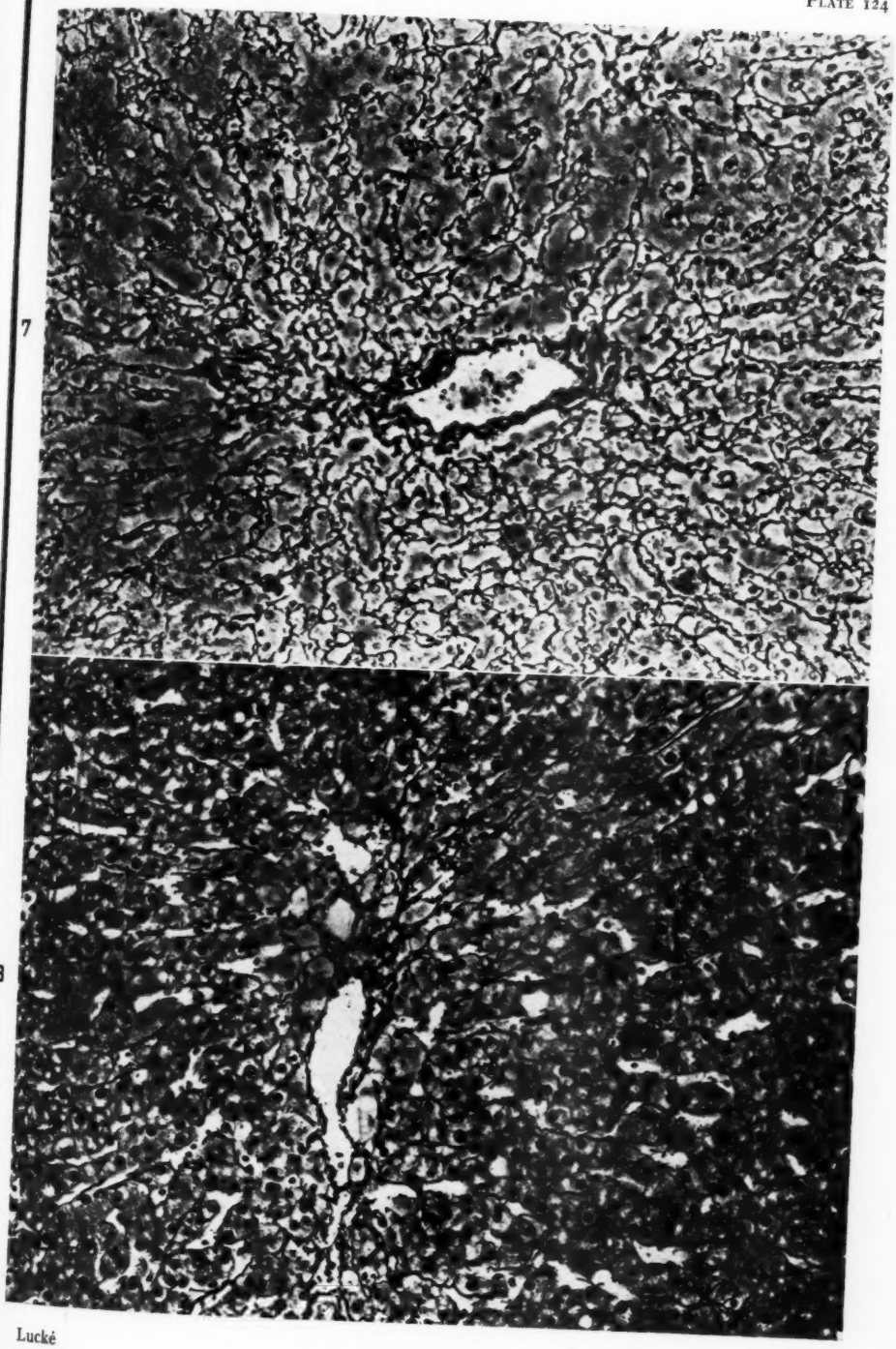
Lucké

Recovery from Epidemic Hepatitis

PLATE 124

FIG. 7. Case 6. Central part of a lobule showing efferent vein, converging columns of liver cells and reticulum frame. The pattern of arrangement is normal. Restoration of this liver is practically complete. Wilder's reticulum stain. × 275.

FIG. 8. Case 11. Hepatic lobule, showing normal pattern of arrangement of cell cords and reticulum. Restoration of structure is complete. Wilder's reticulum stain. × 250.



Lucké

Recovery from Epidemic Hepatitis

SARCOIDOSIS OF THE SPLEEN

Report of a Case with Autopsy and a Study of Intracellular "Asteroid Bodies" *

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Although Boeck's sarcoid of the spleen, as a more or less localized form of the disease, is described under that title in no previously published autopsy report, it undoubtedly is of occasional occurrence and should be included in the differential diagnosis of splenomegalies of obscure origin. In the literature there are several clinical case reports of splenomegaly caused by sarcoidosis, often unsuspected until discovered histologically, and it is probable that some autopsy reports of this disease have been published under another diagnosis, as I shall indicate later.

The Spleen in General Sarcoidosis

Involvement of the spleen in the course of generalized sarcoidosis is a fairly regular occurrence, as proved by both clinical and post-mortem data. Thus, Palmer[1] stated in his review, "of the abdominal signs, splenomegaly is the most prominent," and he added that slight splenic involvement is quite common and that occasionally the enlargement may be very great. Another example of this statement is found in the recent report of clinical data on five cases of Boeck's sarcoid, by Bernstein and Oppenheimer.[2] One spleen extended four fingersbreadth below the costal margin and had a palpable notch, a second spleen extended 2 cm. below the costal margin, a third extended 1 cm. below the costal margin, a fourth just reached the costal margin, and the fifth was not palpable. Of Nickerson's[3] six patients, four had splenomegaly, with spleens weighing 200, 230, 640 and 890 gm. respectively. He stated that splenomegaly, with or without lymphadenopathy, was the most constant feature of his six cases.

Histologically, the spleen is the second most common site of involvement in generalized sarcoidosis, as shown in Table I. (The data have been collected from Pinner's[4] review of all autopsied cases up to 1938 and from twelve subsequent case reports,[5-12] including my own. It is incomplete since in a few cases only partial autopsies were performed, while in others many tissues were not examined histologically. However, it provides a fairly correct picture of the relative incidence in the various tissues. The case of Ronchese[13] and the sixth case of Bern-

* Received for publication, July 6, 1943.

stein and Oppenheimer[2] are not included in this anatomical summary because they show too much evidence of ordinary tuberculosis.) In view of the affinity of sarcoidosis for the reticulo-endothelial system, it is not at all surprising to find this high incidence in the spleen; nor is it surprising to observe that nearly all of the cases examined at autopsy show a widespread rather than a localized distribution of the disease, even though most of the patients had died of causes other than sarcoidosis itself.

TABLE I

Incidence of Tissue and Organ Involvement in 29 Cases of Sarcoidosis Examined by Autopsy

Tissue or organ	Number of times involved
Lung	24
Spleen	21
Lymph nodes	21
Liver	17
Bone marrow	14
Skin	12
Kidney	7
Gastrointestinal tract	5
Trachea and bronchi	5
Tonsils	3

Other tissues reported to be involved 1 to 6 times are: heart, pituitary body, brain, spinal cord, meninges, prostate, testicle, epididymis, thyroid, pancreas, skeletal muscle and nasal mucosa.

Occurrence of Localized Sarcoidosis of the Spleen

In spite of this high incidence of splenic involvement in the generalized form, sarcoidosis of the spleen as a disease in its own right, with only insignificant or absent sarcoid lesions elsewhere, is but little known and therefore is usually unrecognized even when it does occur. The "pure" form, with no lesions outside of the spleen, is probably nonexistent, and the other cases with splenomegaly and vague symptoms predominant in the clinical picture have usually been mis-diagnosed. Thus, Palmer[1] referred to enlarged spleens which were removed for "Banti's disease" and then found histologically to show sarcoidosis instead, and he therefore urged consideration of sarcoidosis in every case of chronic splenomegaly. In Jadassohn's[14] third case, for example, the patient presented typical sarcoid lesions in skin and bones, responded negatively to the Pirquet and Mantoux (1:1000) tests for tuberculosis, and had had splenectomy for Banti's disease 3 years previously; the spleen showed sarcoidosis. In one of Longcope's[9] cases of sarcoidosis, splenectomy was done for supposed Banti's disease and the diagnosis of sarcoid made after histological examination of the spleen. In Engelbreth-Holm's[15] second case, in which splenectomy for supposed Banti's disease was performed, the patient responded nega-

tively to the Mantoux skin test for tuberculosis, showed no acid-fast bacilli in the spleen, and showed multiple, tiny, noncaseating tubercles in the spleen. Although the case is reported as tuberculosis of the spleen, this evidence is against tuberculosis and very strongly in favor of sarcoidosis, as will be discussed later.

Dressler's[16] case of unexplained splenomegaly was diagnosed as sarcoidosis of the spleen on the basis of sarcoid lesions having been found in a sternal puncture, on roentgenological findings suggestive of hilar sarcoidosis, and on negative response to the Pirquet test and only weakly positive response to the Mantoux test (using 1 mg. of tuberculin). Dressler concluded that many cases diagnosed as Banti's disease or as tuberculous splenomegaly are actually sarcoidosis. That the possibility that sarcoidosis is a cause of splenomegaly of "obscure origin" is seeping into current clinical thought, is demonstrated by a recent clinicopathological discussion,[17] involving a patient whose spleen reached nearly to his left iliac crest. His negative response to the Mantoux test for tuberculosis, the elevation of total serum protein, and the hyperglobulinemia were the only signs of sarcoidosis. This diagnosis was offered clinically, however, even before the pathologist's presentation, to the group, of sarcoid lesions in specimens of liver and eyelid removed for biopsy. (The spleen was not removed and the patient was living at the time of publication of that paper.)

Tuberculous Splenomegaly

"Primary" tuberculosis of the spleen, as opposed to the splenic lesions accompanying active tuberculosis elsewhere in the body, is accompanied by only minor tuberculous lesions elsewhere, usually in the abdominal lymph nodes, the liver and the lungs. As described by Winternitz,[18] this is usually a severe and often fatal disease, and therefore I believe that patients with mild symptoms or with no symptoms at all should be regarded with suspicion until the diagnosis definitely has been proved. Many of the more recently reported cases have had such "peculiarities" as negative skin tests for tuberculosis, absence of tubercle bacilli in stained sections and even in inoculated guinea-pigs, absence of definite fever or of any severe symptoms, presence of only noncaseating tubercles, and evidence of active or healed "miliary tuberculosis" of the spleen with only mild symptoms and with recovery. It hardly need be stated that each of these items is a point in favor of sarcoidosis and against ordinary tuberculosis. In fact, a truly negative tuberculin skin test reaction in a patient with mild symptoms and noncaseating tubercles makes the diagnosis of sarcoidosis almost mandatory; and such occurrences as "healed miliary tuberculosis," or the

failure of tissue with *active* lesions to infect a guinea-pig, speak very strongly against tuberculosis but are the customary findings in sarcoidosis. Let us now examine some individual cases.

The earlier review by Winternitz[18] contributes nothing definite to our search for cases of splenic sarcoidosis. The majority of his patients died as a direct result of splenic tuberculosis or its generalized dissemination, they had caseation in the splenic lesions, and undoubtedly were straight-forward cases of tuberculosis. Only a small number of his cases had miliary, noncaseous tubercles; a tuberculin test was reported in only one case (positive); and insufficient data are given to label any individual case even as "possible" sarcoidosis. Most of the cases mentioned by Lubarsch[19] either had caseous tubercles or had insufficient data, but one of his cases is very suggestive of sarcoidosis. This patient had a chronic course with splenomegaly and lymphadenopathy, and the spleen was removed surgically and found to have small, miliary, pinhead-sized, noncaseating tubercles with a definite reticulum framework in each one. (This reticulum framework differentiates sarcoidosis from tuberculosis, according to Nickerson,[3] and others.) A guinea-pig injected with splenic tissue from this patient was alive 2½ months later. To explain this apparently benign miliary tuberculosis, Lubarsch postulated a very low virulence for the tubercle bacilli in this case, which is one of the theories that have been offered for the explanation of sarcoidosis itself. (This patient is stated to have died of pulmonary tuberculosis some years later, after a healthy, asymptomatic interim; and although we might be tempted to consider the tuberculosis a separate disease in this case, perhaps even acquired during possible sanatorium treatment for his "tuberculous splenomegaly," nevertheless the mode of death forces us to doubt the classification of this case as Boeck's sarcoid.)

The report by Engelbreth-Holm[15] of 28 cases of tuberculous splenomegaly subsequent to Winternitz's[18] review presents rather hopeful material. His own second case (previously mentioned), with a negative response to the Mantoux test, was diagnosed clinically as Banti's disease and showed noncaseating tubercles and absence of tubercle bacilli in the surgically removed spleen; the patient was still living and well at the time of the report. His third case responded negatively to the Pirquet test for tuberculosis, had miliary noncaseating tubercles in the spleen, and showed a connective tissue framework in the tubercles. In his fourth case the spleen contained noncaseating tubercles and no acid-fast bacilli. Of the 28 cases he reviewed (including his own 4 cases), 3 had Pirquet tests done, and all three were negative. (All

were in cases with noncaseous tubercles.) Of the 9 cases reported as showing acid-fast bacilli in the tissue, 4 had miliary tubercles and 5 had larger caseous ones.

Since it would be unfair and inaccurate to use published statements and photographs to re-diagnose cases of other writers, I can offer no concise list of cases reported as tuberculous splenomegaly which should be labeled sarcoidosis; but I feel certain, for reasons already enumerated, that many such cases exist. The case presented herein is believed to be the first case of splenic sarcoidosis, with autopsy, to be reported under that diagnosis. The disease was asymptomatic, was accompanied by mild splenomegaly and with only a few, small, scattered, sarcoid lesions in other tissues, and was a purely incidental finding in a woman who had died of meningococcal meningitis. This case has also provided an unusual opportunity to study certain rather rare inclusion bodies which have been previously described in cases of sarcoidosis.

Report of Case

M. E. S. was a white woman, 63 years old, who had visited the medical outpatient department for sundry complaints during the past 5 years.

Past History. About 4 years previously her systolic blood pressure was reported to be 270 mm. of Hg by her family physician. A fairly complete study in the clinic 2 years previously had revealed complete heart block with a very slow pulse rate, evidence of aortic stenosis and very slight cardiac enlargement. At that time there was no enlargement of her lymph nodes. The total serum protein level was 6.7 gm. per 100 cc., and other chemical studies, blood counts, urinalyses, and the spinal fluid colloidal gold curve revealed nothing abnormal. Wassermann tests on blood and spinal fluid were negative. She had had weakness in the 6 months preceding her final entry, and had lost 10 pounds in weight in the past 2 months. Dyspnea had been gradually increasing during the past 2 years.

Present Illness. The patient was brought to the hospital in a semicomatose condition on January 4, 1943, and the history was obtained from her brother. She was said to have developed malaise and epigastric pain and to have vomited black material 2 days before entry. She improved on the following day, then suddenly became very ill, began to "scream with pain," became confused and glassy-eyed, and showed "red marks" on her chest.

Physical Examination. The patient was completely confused, showed rigidity of the neck and positive Kernig and Brudzinski signs, and had a macular petechial skin rash. Her eyes were fixed in a glassy stare. The blood pressure was 150 systolic and 80 diastolic, the pulse rate was 60 per minute, and there were systolic murmurs at the mitral and aortic valve areas.

Laboratory Findings. The white blood cell count was 24,000 per cmm. (about 90 per cent polymorphonuclear leukocytes), and the spinal fluid contained 2000 polymorphonuclear leukocytes per cmm., with intracellular and extracellular gram-negative diplococci.

Course. The patient rapidly became stuporous and died within 5 hours of entry.

Clinical Diagnoses. Meningococcal meningitis; chronic rheumatic endocarditis with aortic stenosis.

REPORT OF AUTOPSY

The autopsy was performed 14 hours after death.

Gross Findings

Skin. There were numerous small petechiae on the skin of the abdomen, the thorax and most of the flexor surfaces.

Heart. The heart weighed 500 gm. and showed thickened, fibrotic, partially calcified aortic valve cusps, which were partially fused and had caused marked stenosis of the valve. The mitral valve had thickened, fibrous, partly calcified leaflets and was slightly stenosed; the chordae tendinae were short and thick. The left ventricle was hypertrophied and measured 1.7 cm. in thickness.

Lungs. The lungs were congested and mildly edematous.

Spleen. The spleen weighed 300 gm., had a smooth red capsule, was slightly softened and showed on its cut surface a myriad of tiny white nodules 0.3 to 1.0 mm. in size, which were embedded in a soft red pulp. They could not always be distinguished grossly from malpighian corpuscles, although they were thought to be a trifle smaller and were discrete and circumscribed. No caseation nor large nodules were seen.

Lymph Nodes. No enlargement nor gross abnormality.

Liver. The liver was moderately congested. The *gallbladder* had a thin, tough, white, fibrosed wall and was completely filled by a large, rough, brown calculus.

Brain. The brain weighed 1360 gm. and had a moderately thick, diffuse, subarachnoid purulent exudate which was most abundant on the surface of the cerebral hemispheres.

Histological Findings

Spleen. The spleen was studded with discrete, circumscribed, tubercle-like lesions which were all of fairly uniform size (slightly smaller than a malpighian corpuscle) and located indiscriminately in the lymphoid tissue and sinusoidal areas. Although they sometimes overlapped, the lesions tended to retain their individuality rather than to become confluent. The individual lesion was elliptical or spherical, and had a smooth and sharply outlined border and a reticulum and collagenous connective tissue framework in which the fibers tended to radiate from center to periphery. There was a narrow zone of concentrically arranged reticulum fibers at the edge of each sarcoidal lesion. Endothelioid cells and multinucleated giant cells of various sizes were embedded in the pale-staining collagenous connective tissue, and these were sometimes oriented toward the center of the nodule.

In the larger giant cells there was a tendency for the nuclei to become bunched together at one end, while the cytoplasm forming the bulk of the cell became the site of certain striking processes. In most giant cells the cytoplasm showed a few or many punched-out clear areas, often with a tiny, pink-staining sphere or coccoid body in the center of each such vacuole. The larger vacuoles contained larger spheres, some of which had short, sharp pseudopodia or rays protruding from their surfaces. In other cells these rays had become much longer, additional rays appeared, and the structure increased in size until it reached the maximum size observed (about 25 μ). At this stage there was rarely more than one such structure in any single cell, although many other "embryonic" vacuoles might be present, and the large parent vacuole had become very irregular in order to adapt itself to the shape of the full-sized body with its long rays. This fully developed star-shaped body, to which I shall refer as an asteroid, was somewhat more acidophilic and less granular than the cytoplasm of the giant cell and often had a small, bluish-pink-staining sphere exactly in its center. (The chemical properties are discussed later.) The asteroids were found nowhere except within the cytoplasm of multinucleated giant cells. Only about 6 or 8 per cent of giant cells had fully developed asteroids, although the majority of cells had small vacuoles. The accompanying high-power photomicrographs of various giant cells have been arranged in order of development to illustrate the hypothesis of growth just described.

No acid-fast bacilli were found in sections of spleen stained by the Ziehl-Neelsen technic.

Lymph Node. Section of a small abdominal lymph node near the gallbladder showed numerous, small, discrete collections of endothelioid and giant cells, with very little connective tissue and with no caseation in these lesions. These nodules were smaller and more youthful in appearance than those in the spleen, and their giant cells contained vacuoles and a few asteroids like those previously described.

Lungs. In several sections of lung tissue there was found an occasional, very tiny collection of endothelioid cells and perhaps a giant cell or two with an asteroid. There was some congestion.

Liver. In two large sections of liver there was found a tiny collection of cells similar to those in the lung. There was moderate passive congestion.

Brain. There was some edema of the molecular layer of the cerebral cortex, adjacent to the thick layer of subarachnoid polymorphonuclear exudate.

Other Tissues. Sections of myocardium, pancreas, gallbladder,

adrenal, kidney, ovary, pituitary gland, and abdominal skin showed no evidence of sarcoidosis, nor of any other lesion except chronic cholecystitis. Other tissues were not sectioned.

Unfortunately all of the tissues were placed immediately in fixative and nothing was available for culture or animal inoculation, as the presence of sarcoidosis was not suspected until the slides were seen.

Anatomical Diagnoses

Meningococcal meningitis; rheumatic endocarditis, mitral and aortic, with aortic stenosis and left ventricular hypertrophy and with passive congestion of lungs and liver; chronic cholelithiasis and cholecystitis; sarcoidosis of the spleen and lymph node, with isolated tiny sarcoidal lesions in the lungs and liver.

Staining and Chemical Reactions of Asteroids

Since the asteroids were found only within giant cells of the specific lesions in spleen, lymph node and lung; since I have not seen them in tuberculous or other material treated with the same formaldehyde fixative; since they are present in frozen sections as well as in paraffin sections; and since they are seen with all stains and even in unstained sections, they are presumed *not* to be artifacts and to be definitely part of the sarcoidal lesion. A resumé of their properties, as thus far determined, is as follows:

1. Solubility. Asteroids fail to dissolve in water, alcohol, or xylol.

2. Hydrogen ion concentration. The asteroids neither dissolve nor show any morphological alteration when sections of spleen 5 μ in thickness are immersed in either 1 per cent sodium hydroxide or 10 per cent formic acid for 15 minutes.

3. Fat stains. With scarlet red or Sudan III the asteroids remain unstained; some macrophages and a few giant cells contain small globules of neutral fat, but no fat is found in the specific vacuoles of the giant cells. With Nile blue sulfate asteroids are dark blue. With Fischler's fatty acid stain asteroids are very faintly salmon colored (negative).

4. Hematoxylin and eosin. Asteroids are pink.

5. Phosphotungstic acid hematoxylin. Asteroids are vaguely bluish or sometimes pale orange.

6. Mallory's connective tissue stain. Asteroids are reddish violet (about the same as cytoplasm of cells), negative for collagen.

7. Masson's trichrome stain. Asteroids are gray (negative for collagen).

8. Hematoxylin and van Gieson's stain. Asteroids are yellow (negative for collagen).

9. Wilder's reticulum and van Gieson's stains. Asteroids are pale pinkish yellow (negative for reticulum).

10. Weigert's elastic tissue stain. Asteroids contain no elastic tissue (nor does the rest of the sarcoidal lesion).

Conclusion. The asteroids are relatively stable chemically; they respond negatively to the more common stains for specific substances, and they exhibit approximately the same staining reactions as the cytoplasm of endothelioid or giant cells.

Discussion

Such asteroids have been known for some time in connection with sarcoidosis, and Jadassohn,[14] in 1919, referred to "asteroid bodies" of unknown microchemical identity in his first case. They are rarely mentioned in the more recent literature on sarcoidosis, however, probably because they occur infrequently (judging by the other sarcoid material in this laboratory).

A type of inclusion body which was not found in this case but which is commonly described in sarcoidosis and which has been exhaustively studied by Schaumann[20] is roughly spherical or oval-shaped, often has concentric laminations, may show yeast-like budding, is nearly always calcified, and is usually large and often much larger than its parent giant cell. Some of these bodies show rays or fascicles, suggesting that they may be an older, more chronic, calcifying stage of the asteroid. Schaumann contended that these calcifying bodies may represent tissue reaction around nonbacillary, fungoid or mycotic forms of the tubercle bacillus, and that this nonbacillary form of the organism may be the etiological agent of sarcoidosis. Although the asteroid may possibly be a less mature form of the calcifying inclusion body, I can offer no evidence from this case to prove any relation between the asteroid and the tubercle bacillus (nor any evidence against such relationship).

Wolbach,[21] in 1911, described intracellular bodies exactly similar in morphology and most staining reactions to these asteroids. These were found in 5 cases in a series of 900 autopsies. The patients had died of such diverse diseases as malignant neoplasms and pernicious anemia, and following thyroidectomy. He therefore concluded that these bodies were nonspecific. This study was completed before the general recognition of Boeck's sarcoid as a disease entity, and Wolbach's description of these bodies in noncaseating "tubercles" which tended to undergo fibrosis is very strongly suggestive of sarcoidal lesions; his painstaking

care to rule out tuberculosis as the cause of the tubercles is further evidence of this point. The diversity of the principal diseases in the 5 patients detracts nothing from the possibility of their having sarcoidosis, as most of the published autopsy reports of cases of sarcoidosis state that some other disease was the cause of death. An interesting point is the combined distribution of lesions in Wolbach's 5 cases (lung, lymph nodes, liver, spleen), which is precisely the distribution in my single case.

Wolbach's [21] conclusions that the asteroid body is nonspecific, is not a parasitic organism and is a biochemical alteration of cytoplasm might be modified in the light of present knowledge to the following:

1. The asteroid *may* be nonspecific but is characteristically found in sarcoid lesions.

2. The possibility of its being an extraneous organism cannot be excluded, although it is doubtful.

3. Its causation and its chemical or histological identity are still unknown, specific tissue stains give negative results and it does not exhibit the features of a crystalline body.

Summary

1. Sarcoidosis of the spleen is an occasional clinical condition which should be considered in cases of obscure splenomegaly. In the past it has been misdiagnosed most often as Banti's disease or as tuberculous splenomegaly.

2. In the case presented, sarcoidosis was found at autopsy to be primarily splenic. In this instance sarcoidosis was asymptomatic and was an incidental finding in a patient who died of meningococcal meningitis.

3. Intracellular asteroid bodies were present in the sarcoidal lesions. Investigation by histochemical methods failed to give definite information as to their causation or histological identity.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

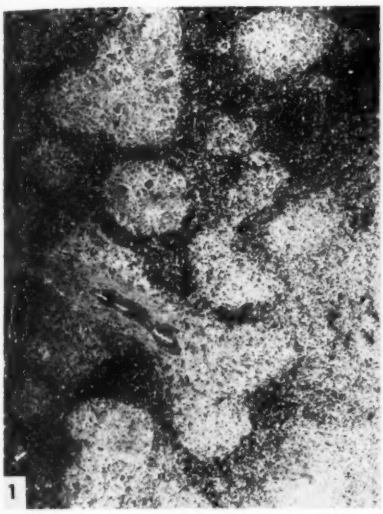
PLATE 125

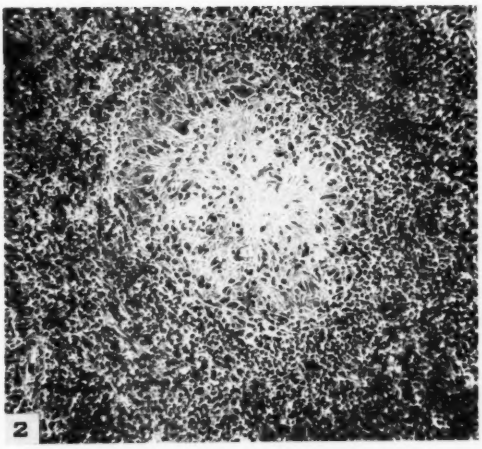
FIG. 1. Sarcoid nodules in spleen, showing smooth, sharp borders. Hematoxylin and eosin stain. × 30.

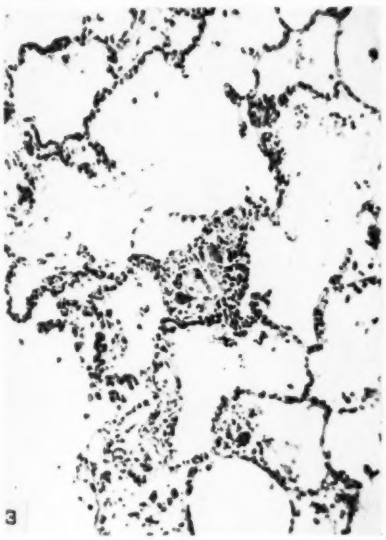
FIG. 2. Single nodule in spleen, showing one giant cell and much fibrosis. Hematoxylin and eosin stain. × 90.

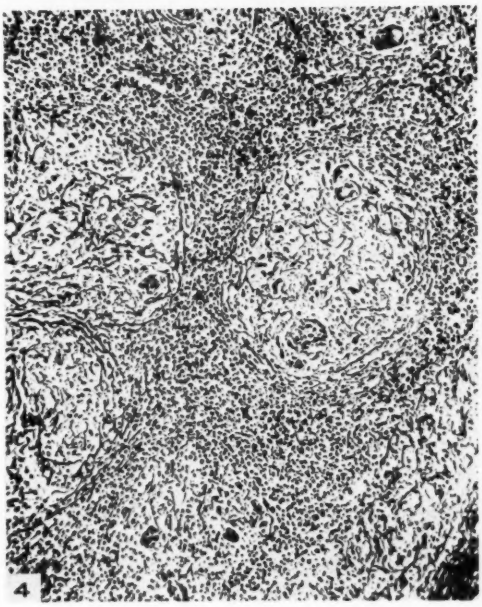
FIG. 3. Typical, but relatively insignificant, collection of a few giant and endothelioid cells in lung. Hematoxylin and eosin stain. × 90.

FIG. 4. Reticulum framework of sarcoid nodules in spleen. Wilder's reticulum stain. × 90.









Friedman Sarcoidosis of the Spleen

PLATE 126

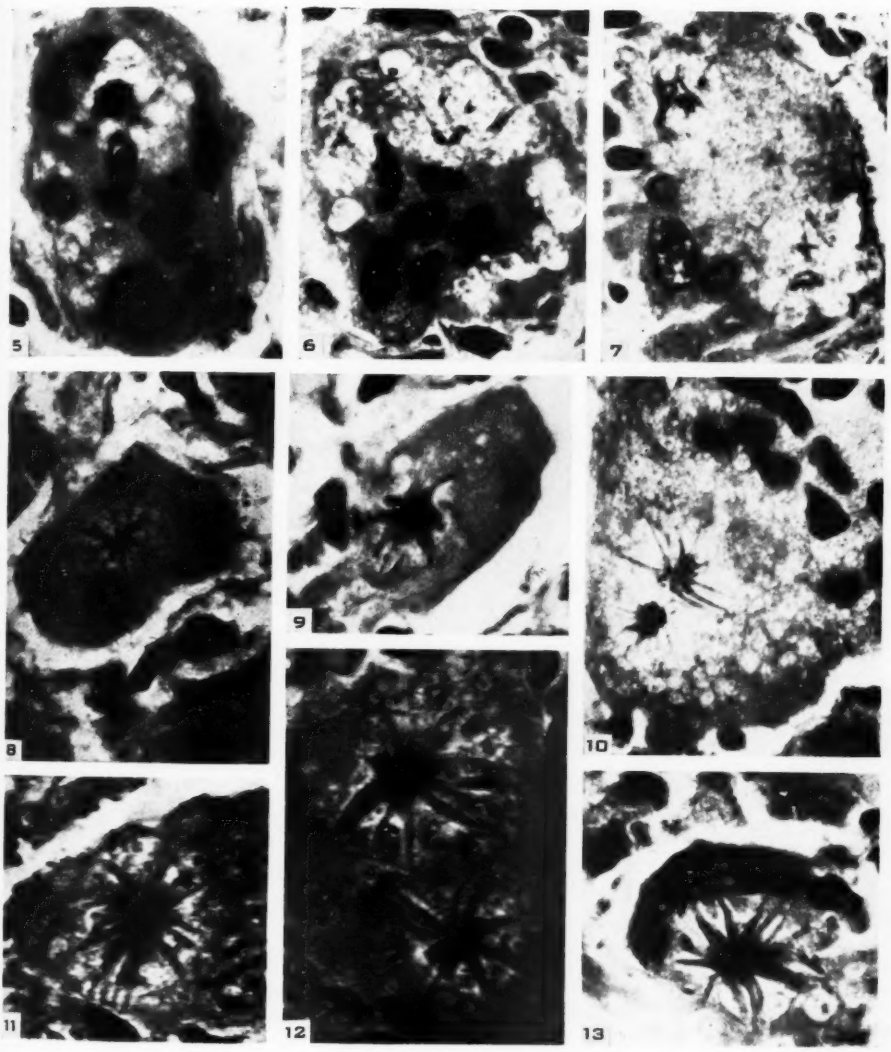
(All figures are from the spleen)

FIG. 5. Giant cell with early vacuole formation. Hematoxylin and eosin stain. × 795.

FIGS. 6 and 7. Several coccoid bodies have tiny irregular spicules. Hematoxylin and eosin stain. × 795.

FIGS. 8, 9 and 10. Asteroids of intermediate size; numerous coccoid bodies are present in small vacuoles in Figure 10. Hematoxylin and eosin stain. × 795.

FIGS. 11, 12 and 13. "Fully developed" asteroid bodies in giant cells. Hematoxylin and eosin stain. × 795.



Friedman Sarcoidosis of the Spleen

HISTOLOGY OF CEREBRAL LESIONS PRODUCED BY FOCUSED ULTRASOUND*

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Ultrasonic waves, sometimes called supersonic waves, are mechanical or elastic vibrations in any solid, liquid, or gaseous medium of a frequency above the range of audible sound, namely, above 16,000 cycles per second. Although the physical laws of audible sound also apply in the higher supersonic frequencies, the latter exhibit additional phenomena not found in the audible range. These special phenomena are a result of the high frequency and short wave length. Ultrasonic waves are, for example, capable of producing destructive changes in living tissue. Some of these changes are: mechanical "trip hammer-like" beating with rupture of the tissues, heat generation and sudden explosive liberation of dissolved gases from solution in the irradiated tissues with a resulting cavitation which resembles a localized caisson disease. Finally, ultrasound has the property of being focusable within a small spot a few millimeters in diameter. This property of focusability, in conjunction with the special tissue effects, suggests the possibility that an optimum combination of wave length, intensity and exposure might be found which would produce a focal destruction in the depths of tissues, without injuring the intervening layer. While this possibility has not been realized in the present experiments, it has been approached.

Since Harvey, Harvey and Loomis,[1] in 1928, conducted their pioneering experiments on the biological effects of ultrasound on living microorganisms and animals, a host of domestic and foreign workers have extended its biological and industrial applications. At frequencies above 500 kilocycles, the supersonic waves are produced by driving a quartz crystal to expand and contract along its x-axis with an alternating current which is tuned to the same frequency as the natural frequency of the crystal.

In 1935 the physicist, Gruetzmacher,[2] ground the surfaces of a vibrating quartz plate so that they had a curve similar to that of a concave mirror. This caused the ultrasound waves thrown off to converge and come to a focus. In so far as we are aware, the present series of

* This report was presented in condensed form under the title of "The Use of Focused Ultrasound Waves for the Production of Transcranial Focal Brain Lesions: a Preliminary Report" before a combined meeting of the New York Neurological Society and the Section on Neurology and Psychiatry of the New York Academy of Medicine, December 8, 1942.

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studies represents the first biological applications of this new curved quartz plate method of obtaining local concentrations of ultrasonic energy. In the experiments herein reported we have used a quartz plate generator with the following specifications: round, diameter 5.08 cm., frequency 835 kc., curved to focus at a spot 5.5 cm. from the concave crystal surface or 4 cm. above the center of the flat cellophane diaphragm. The focal spot in water has the same diameter as the wave length, namely, 1.8 mm. Technical details regarding the construction and operation of both the radio-frequency and the focusing ultrasound generators have already been reported.[3]

Material and Procedures

In all, 3 dogs, 30 cats and 4 monkeys were used. The animals were all under ether anesthesia. Various areas of their heads or backs over the spinal column were shaved free of hair and then placed against the cellophane diaphragm of the focusing ultrasound generator in the manner seen in Figure 1. An ultrasound transmission medium of a few millimeters of olive oil always had to be used between the skin and the diaphragm, taking care that no bubbles of air were present anywhere between the source of ultrasound and the skin surface, since the presence of air bubbles absorbs and disperses the radiations.

All except the last of the 37 experimental animals were treated from 5 to 15 minutes with maximum doses of focused ultrasound. Our overcautious attitude towards the danger of breaking the quartz caused us routinely to increase very slowly the voltage applied to the crystal generator. It usually took 30 to 60 seconds for the intensity of the striking force of the focused ultrasound to be increased from 0 to peak output, at which level it could be maintained with safety for the crystal during the remainder of the treatment period.

Behavior and Structural Changes Following Application of Focused Ultrasound to the Nervous System

The most characteristic deviations in behavior which were produced were blindness after transcranial irradiation of the striate region of the occipital cortex, ipsilateral incoordinations and ataxia from an application to one cerebellar lobe, bilateral paralysis and anesthesia of the hind extremities following treatment of the lumbar cord region, and monoparesis of the right forelimb after irradiation of the forelimb area of the contralateral motor cortex.

Immediately after a treatment of 3 minutes or longer at maximum intensity the skin had a slightly yellowish tinge, probably due to the contact medium of olive oil having been driven into the pores by the

radiation pressure. Swelling and edema of the skin and underlying soft tissues did not become manifest for 10 or 15 minutes and then persisted for 3 or 4 days, during which time the skin gradually turned a brownish gray. By the end of 8 or 9 days the edema had disappeared but the skin was dead and leathery in consistency and appearance. It was sharply demarcated from the healthy skin by an inflamed margin from which it could be detached. By this time, the underlying soft tissues and muscles were markedly blanched, cold, and free from any signs of circulation or edema. In fact, they were even shrunken and atrophic, and the muscle cells showed disappearance of their nuclei and fading of cellular outlines. The round area of necrotic muscle was as sharply demarcated from healthy muscle as was the treated area of overlying skin from the healthy skin.

By the end of 12 days, the affected area presented the typical depressed, shrivelled and hornified appearance of dry gangrene (Fig. 2). Except for a slight yellowish discoloration of the periosteum, the skull and vertebrae showed no signs of injury. This was also true of the dura mater, except in two animals which exhibited epidural and subdural hemorrhages respectively. The nervous system always seemed to be damaged when the thinness of the overlying superficial soft tissues permitted the transmission of a sufficient concentration of the ultrasonic radiations. The character and location of this neural damage will be described in the representative protocols of experimental animals. These protocols include descriptions of the abnormalities of behavior produced in association with specific neural lesions. All animals, with the exception of monkey 4, were killed for autopsy by injecting 30 to 40 cc. of an 8 per cent solution of formaldehyde into the heart. After gross pathological changes had been noted, the brains and cords were fixed in a 4 per cent solution of formaldehyde, imbedded in paraffin and sectioned and stained by Nissl and silver impregnations.

Four animals will be described briefly as representative of the 37 animals used in this series. They give ample evidence of the functional and structural changes produced in the nervous system by focused ultrasound.

Cat 56: Transcranial Application to Occipital Striate Area

The cat became totally blind with recovery 4 hours later. Autopsy at this time showed edema and inflammation of cortico-striate regions on both occipital lobes.

The animal was a healthy male cat, weighing 4.1 Kg. After the routine preparations the occipital midline region of the head was placed against the diaphragm of the ultrasonic generator. The cat was given a 15 minute treatment at peak ultrasonic output level (radio-frequency current, 0.85 amperes).

One hour after treatment the animal was completely out of the ether anesthesia. The scalp was edematous over the area treated. He crawled slowly about the floor with perfect coordination but with his nose and belly touching the ground. He bumped his nose repeatedly against the walls and furniture. This invariably produced an immediate rapid backward withdrawal followed by a change in the direction of his creeping progress. Waving a hand in front of his eyes, or flashing a light into them, produced no distraction of attention and no change from the progressive belly crawling and nose-down position. Both pupils contracted equally to light. Clapping of the hands or ringing a bell caused pricking up of the ears and a momentary swinging of the head in the direction of the sound.

Four hours after exposure to ultrasound the cat walked normally, erect, avoiding with ease all objects in his path. The only behavioristic deviation observed at this time was a marked photophobia. Flashing a light into his eyes produced many meows in association with a precipitous flight to a shady corner.

Pathological Findings. Four and one-half hours after treatment the animal was killed and autopsied in the standard manner. The scalp showed the usual area of severe damage which appeared as a round, darkened, edematous patch about the size of a twenty-five cent piece. A trephine opening was made through the skull in this region, and the brain and meninges tended to bulge outward through it. They showed dilated blood vessels. The underlying occipital cortex for about 1 cm. bordering both sides of the posterior one-third of the longitudinal sulcus was moderately edematous with an ironing out of convolutions and sulci. The remainder of the brain and meninges appeared normal.

Monkey 2: Transcranial Application to Arm Area of Right Motor and Premotor Cortex

This monkey had a persistent spastic paresis of the left upper extremity which underwent only a partial recovery after 12 days, at which time autopsy showed a well circumscribed focal lesion in the right motor and premotor cortical arm area.

Monkey 2 was a healthy male animal which, after the usual preparations, was given a 10 minute dose of focused ultrasound at maximum intensity to the head over the right motor and premotor cortical arm areas.

One hour after treatment, he was completely out of anesthesia, leaping and climbing about carrying his left upper extremity in a semiflexed spastic position (Figs. 3 and 4). He succeeded in using it only very little, mostly at the shoulder and elbow when climbing and eating. Grasping of food and objects was done largely with the right hand. The left hand pushed slightly but could not grasp. The left arm muscles were spastic, with hyperactive deep reflexes. He could not support his weight with his left hand and arm.

Twelve days after ultrasonic application, the animal showed a typical dry gangrene of the soft tissues of the scalp overlying the region irradiated (Fig. 2). However, there was a return of much of the use of the left hand and arm, as shown by greater use in eating and climbing, with improved grasping and weight-supporting ability. Despite this improvement there was still an obvious residual paresis associated with manual clumsiness, a hyper-reflexia, and tendency to carry the member in a semiflexed position.

Pathological Findings. On the afternoon of the 12th day after treatment, the animal was killed and autopsied according to the usual procedure. Grossly the brain exhibited areas of old pial hemorrhagic discoloration over the middle portion of the right motor and premotor cortical regions. These even extended into the adjacent frontal and post-central areas (Fig. 5). Microscopically, this region

showed the usual clearly defined cone-shaped region of necrosis with the base at the surface of the cortex. On cross section the necrotic area appeared triangular (Fig. 6), and was walled off from the normal cortical tissue by a heavy layer of glial and blood vessel proliferation. In the severely affected region there were barren acellular areas where only an occasional ganglion cell remained. These showed swollen, tortuous, broken or missing processes. Figure 7 illustrates such a nerve cell with its apical dendrite swollen and corkscrew-shaped.

Cat 153: Transcranial Application to Left Cerebellum

The cat exhibited a marked ataxia, incoordination and weakness of his left hind extremity, without, however, any sensory disturbance. This lasted over an hour and was followed by an apparent complete functional recovery, despite subsequent morphological evidence of persistent damage to the ganglion cells of the left cerebellar cortex.

Cat 153 was a healthy male animal, weighing 3.8 Kg. After the usual preparations the left cerebellar region was given 15 minutes of irradiation at maximum intensity.

One hour afterwards the animal was completely recovered from the ether anesthesia but walked with hind legs forming a wide base. The skin and soft tissues over the cerebellar region treated were darkened and edematous. The left rear extremity was weak, unsteady and ataxic when walking. Stepping on the animal's tail directly posterior to its body as it lay on the floor caused a violent struggle to escape, with marked deviations to the left on each of eight trials. Alternate pin-pricking of right and left fore-shoulders produced successful avertive movements to the left only, in all of ten trials. When the animal was dropped from a height of 4 feet, the left hind extremity collapsed and precipitated the cat to the floor.

Two hours after the ultrasonic treatment the animal was completely recovered with no signs of ataxia or weakness. On falling, it landed normally on all four legs.

Pathological Findings. Two days after treatment the cat was killed and autopsied in the usual manner. The skin and soft tissues at the site of treatment were beginning to undergo necrosis. A small area the size of a dime on the surface of the left cerebellar hemisphere appeared "moth-eaten." Microscopical examination showed a cone-shaped region of massive destruction of cerebellar tissue lying below this surface area. Adjacent regions of the cerebellum showed no damage (Fig. 8).

Monkey 4: Transcranial Application to Left Cerebellum with Sudden Death

Contrary to the usual practice, *instantaneous* full power of focused ultrasound was applied over the left cerebellum of monkey 4. The animal died within 45 seconds.

The animal was a healthy male. He was placed with his shaved left cerebellar region over the ultrasound generator. The full current of 0.76 amperes was applied *instantaneously* to the crystal with the production of an equally sudden peak intensity of application of the focused ultrasonic radiations to the monkey's left cerebellum and brain stem. Within 10 seconds, the animal was breathing irregularly and in 45 seconds his breathing had ceased. At this time the ultrasound was turned off. All attempts at resuscitation were useless. The quartz crystal was found to have been fractured. An autopsy was done immediately.

Pathological Findings. A small, round, slightly "moth-eaten" area was present on the surface of the left cerebellar hemisphere. The overlying skin and superficial tissues appeared normal. A microscopical study of the cerebellum showed a well

defined and well localized cone-shaped region about 1 cm. across the base and extending about a centimeter below the surface of the left cerebellar cortex. This region exhibited acute edema with cavitation and dismemberment of the neuro-architecture. This was especially evident in the molecular layer. The Purkinje cells were distorted and swollen (Fig. 9). The remainder of the cerebellum and brain stem seemed normal. No structural changes were visible which could account for the sudden death of the animal.

Discussion of Findings

As can be seen in the sample animal protocols presented herein, all circumscribed *in vivo* neural lesions produced to date, with the exception of one, have been associated with a severe necrosis of the adjacent scalp and skin areas lying in the path of the ultrasound radiations, although there was no detectable damage to the bone and meninges. It was felt that sufficiently circumscribed brain and cord lesions had been produced transcranially and transvertebrally to justify this early report.

In contrast to the cats and dogs, the monkeys were especially satisfactory experimental animals because of the almost complete lack of radiation-absorbing muscle tissue over the skull areas adjacent to the motor and occipital regions of the cortex, and because of the greater degree of corticalization of function which resulted in the production of more clear-cut and more persistent focal disabilities.

In general, it can be said that the initial effect of focused ultrasound irradiation of maximum intensity, *instantaneously* applied, is characterized by a mechanical tearing of the tissue architecture and the formation of cavities which may or may not be due in part or in whole to the sudden explosive release of dissolved gases from tissue solution, "le phenomène de cavitation,"[4] (Fig. 9). Rapid development of edema with dilatation of capillaries follows immediately. The most vulnerable cells are the ganglion cells. They show the greatest degree of destruction and reaction of degeneration (Figs. 6 and 7). The second most vulnerable structures are the glial cells which, if not destroyed along with the neural elements, are usually stimulated to diffuse proliferative overgrowth (Figs. 6 and 7). The least affected of all the cerebral tissues are the blood vessels, which usually show damage in the regions of tissue interfaces only, such as on the surface of the brain or dura mater. At these sites ruptured capillaries with areas of petechial hemorrhage are sometimes observed (Fig. 5). Within the nervous system *per se* the blood vessels may occasionally exhibit damage, but are usually stimulated so that subsequent capillary proliferation and endothelial thickening are seen throughout parts of treated areas of the brain and cord (Figs. 6 and 7).

Summary and Conclusions

A new method of producing transcranial and transvertebral focal lesions of the nervous system with a focused beam of ultrasound has been developed and employed with partial success in experiments with 37 cats, dogs and monkeys. At the will of the operator, reversible, partially reversible and irreversible disabilities could be produced. Some of these were cortical blindness, cerebellar ataxia, monoparesis, and bilateral paresis of the hind extremities. The central lesion generated, and directly responsible for a disability, was uniformly a roughly cone-shaped area 1 or 2 cm. across at the base and extending about the same depth below the surface of the brain or cord. All surrounding neural tissues were normal. The order of decreasing vulnerability to focused ultrasound of the cerebral or cord structures lying within such a cone-shaped lesion was found to be neuron cells, glia and blood vessels. However, in all experimental animals to date, with one important exception, there was always found associated with the central neural lesion a severe peripheral injury to the skin and soft tissues overlying the brain or cord areas treated.

We wish to acknowledge with thanks the aid of Miss Rosette Spoerri of the Department of Anatomy, College of Physicians and Surgeons, Columbia University, who prepared the histological material.

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[Illustrations follow]

DESCRIPTION OF PLATES

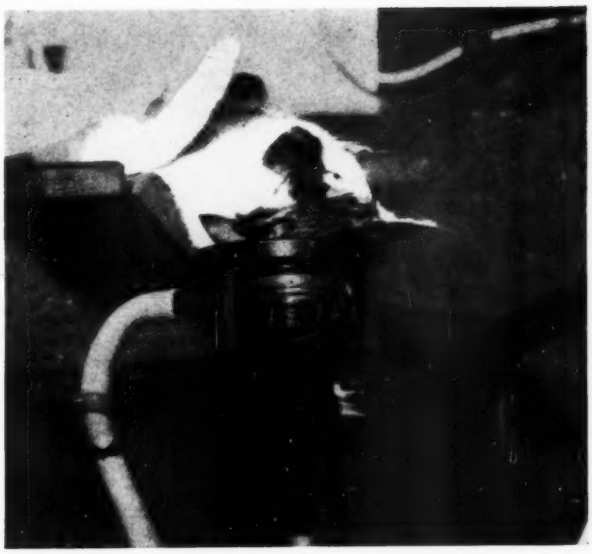
PLATE 127

FIG. 1. The ultrasonic generator in operation with a cat's head in position for irradiation of the left cerebellar region.

FIG. 2. Dry gangrene of soft tissues of scalp on monkey 2, 12 days after a 10 minute exposure to intense focused ultrasound.

FIGS. 3 and 4. Two views of a left spastic monoparesis in monkey 2, 1 hour after a 10 minute transcranial application of focused ultrasound to the right motor and premotor areas.

FIG. 5. An area of subpial petechial hemorrhage on the cortical surface outlines the boundary of the right premotor and motor regions treated. This resulted in the production of the left monoparesis seen in monkey 2, Figures 3 and 4.



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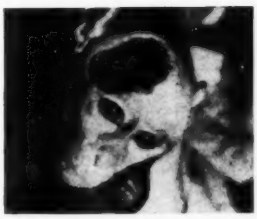

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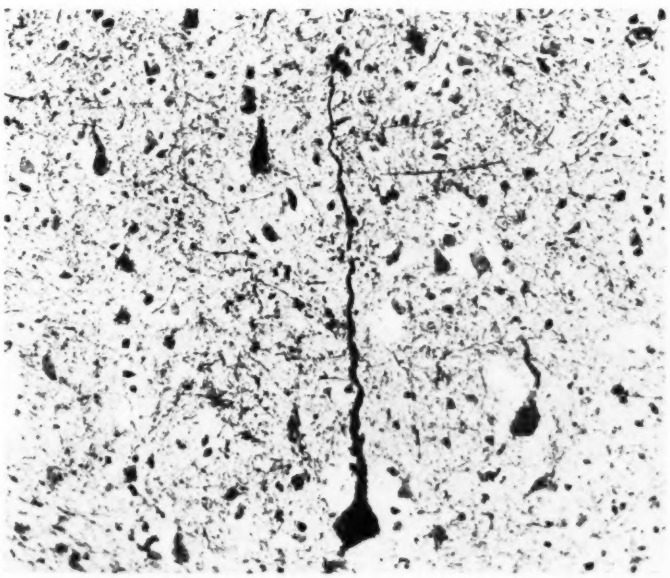
Lynn and Putnam Lesions Produced by Focused Ultrasound

PLATE 128

FIG. 6. Cross section of motor and premotor cortical area of monkey 2. Surface view is shown in Figure 5. The usual cone-shaped region of necrosis is present 12 days after treatment with focused ultrasound. This region is relatively barren and acellular with only occasional atypical ganglion cells remaining. The severely affected region has been walled off from the normal cortical tissue by a zone of glial and vascular proliferation. Modified Nissl's stain. × 50.

FIG. 7. High-power study near the margin of the necrotic region shown in Figure 6. Ganglion cells are scarce and show thickened, broken, tortuous and even missing processes. One large ganglion cell has a swollen and corkscrew-shaped apical dendrite. Silver modification. × 235.

6



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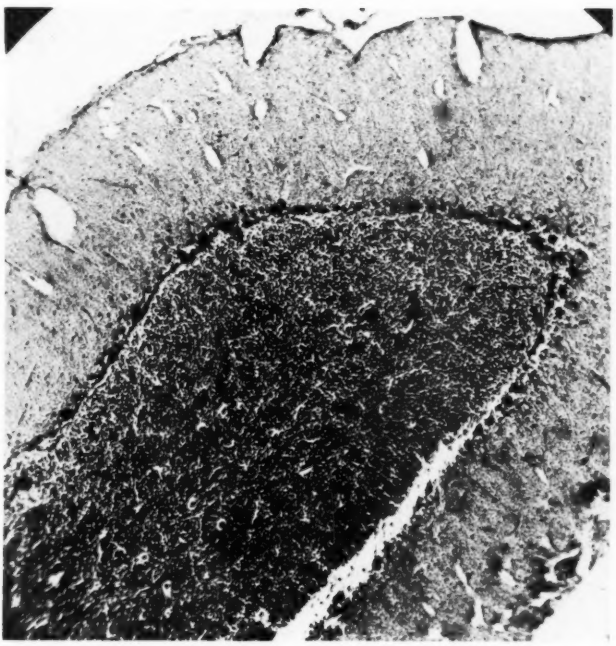
Lynn and Putnam Lesions Produced by Focused Ultrasound

PLATE 129

FIG. 8. Left cerebellum of cat 153, 2 days after 15 minutes of transcranial application of focused ultrasound at maximum intensity. There is a well localized region of massive destruction of tissue near the surface of the hemisphere. Adjacent areas show no damage. Modified Nissl's stain. × 35.

FIG. 9. Leaflet of left cerebellum of monkey 4 immediately after 45 seconds of focused ultrasound was applied transcranially and instantaneously at maximum intensity. Molecular layer is most affected. The tissue has been torn and disrupted, leaving irregular gaps. In addition, diffuse edema exists throughout this layer, while the Purkinje cells show acute distortion and swelling. Silver modification. × 85.

8



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Lynn and Putnam

Lesions Produced by Focused Ultrasound

STUDIES ON THE HEMORRHAGIC AGENT 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN)

IV. The Pathologic Findings after the Administration of Dicumarol *

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It has now been established by many investigators that the administration of Dicumarol to animals and human beings causes a decrease in the prothrombin of blood. This decrease is roughly proportional to the dosage, and death, with or without bleeding, may result from the administration of excessive quantities. Since prothrombin is produced mainly, if not entirely, by the liver, it is a matter of concern whether the liver is damaged by the exhibition of Dicumarol. Before administering the drug to human beings, therefore, we gave it in large doses to 25 dogs at the outset, and subsequently to 4 more. To several of these 29 animals fatal doses were purposely administered. Recently 6 other dogs received Dicumarol in doses approximating those that are now being administered to human beings. It is the purpose of this communication to record our gross and microscopic pathologic findings.

In the early studies on the fatal cases of hemorrhagic sweet clover disease in cattle Schofield[1] reported that gross lobular lesions had been found in the liver. Roderick,[2] however, observed only areas of focal necrosis and these in only half of his cases. He believed that the lesions described by Schofield were due to post-mortem changes. We[3] have previously reported the absence of significant changes in the liver of any dog. Rose, Harris and Chen,[4] however, who have administered large single doses of the disodium salt of Dicumarol to mice, rats and guinea-pigs, and large daily doses for prolonged periods to dogs, rabbits, mice and rats, found central necrosis of the liver in about half the rats examined post-mortem, and occasionally in rabbits, mice and dogs. The rat's liver appeared to be definitely more susceptible to Dicumarol than that of other animals. Five of the 21 dogs that were killed with daily doses of 5 to 50 mg. per Kg. showed central necrosis microscopically, moderate in 1 and slight in the remaining 4.

Procedure and Results

The first 25 dogs received a wide range of dosage: 9 received sublethal oral doses and were killed by shooting, by injection of air or a

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barbiturate into the jugular vein, or by operation with blood loss; 6 received single doses of 35 to 50 mg. per Kg. of the disodium salt of Dicumarol intravenously, and all died without gross evidence of hemorrhage; 2 received single doses of 5 gm. orally, and the remaining 8 received repeated smaller but cumulatively fatal doses over periods up to 7 days. In one instance the total quantity administered in a series of oral doses was greater than 5 gm.

The last 4 dogs of the original 29 were given Dicumarol after we had visited Mr. Rose and Drs. Harris and Chen in Indianapolis in January, 1942, at which time these workers kindly made available their data and animal tissues for study. In these 4 dogs only did we approach the intensive therapy with Dicumarol used by Rose, Harris and Chen,[4] the dosage being 10 mg. per Kg. daily. On this regime 1 dog died on the 8th day after treatment was begun, 1 on the 12th day and 2 on the 14th day.

In none of these 29 dogs, whether given sublethal or lethal doses, was central necrosis of the liver demonstrated. In the animals given large single oral or intravenous doses, death occurred in 3 or 4 to 24 hours without evidence of hemorrhage. Some, but not all, of the animals given sublethal doses showed evidence of hemorrhage at various sites, including the lungs, pleura, gut, and the subcutaneous and intramuscular tissues. In virtually all those that died with hemorrhage, and in many that received sublethal doses of Dicumarol, widespread dilatation of capillaries, arterioles and venules, with degenerative changes, was noted. In the last 4 of the 29 dogs the conditions of administration of the drug were similar to those of Rose, Harris and Chen.[4] Since there were only 4 animals, and since 16 of the 21 studied by Rose *et al.* showed no liver change either, it is obvious that chance alone might account for the fact that we did not make identical observations.

Since no one would deliberately give so large a dose as 10 mg. per Kg. daily to human beings, and since in none of our studies had we given Dicumarol to dogs for as long a period as to patients (up to 90 days), an additional 6 dogs were treated daily or almost daily for an extended period, receiving after the initial dose of 5 mg. per Kg., doses of 1.5 mg. per Kg. When the prothrombin and coagulation times reached levels which made hemorrhage a possibility, the daily dose, as in human beings, was temporarily omitted for a day or longer. In these last 6 dogs the prothrombin and coagulation times were determined semiweekly. In the earlier studies these tests were made triweekly or daily. Three of these 6 dogs were killed by the intravenous administration of 1.5 gm. of nembutal at the end of a month of treatment. One of the remaining 3 dogs died with gross hemorrhage about

the neck after being bitten by another dog. The other 2 were killed after 2 months of observation. Complete post-mortem examinations, including the brain in 2 of the 6 dogs, were done, and detailed microscopic studies of the tissues were made. In this group of 6 dogs no vascular dilatation or other lesion of any of the organs was noted.

Microscopic Findings

The detailed microscopic study of the animals in this experiment included in most instances sections from heart, lungs, liver, spleen, kidney, urinary bladder, stomach, small intestine, colon, pancreas, adrenal, aorta, lymph nodes, bone marrow, areas of gross hemorrhage, and occasionally the brain. The results of this histologic study may be summarized for the first 29 animals, which received large toxic doses of Dicumarol; and separately for the last 6 dogs, which received doses within the therapeutic range.

Hemorrhages. Microscopic hemorrhages were present in almost all of the animals of the first group whether or not they had been revealed in the gross examination. These were irregularly distributed and occurred in almost all the tissues. In most instances, they were capillary or venular and numerous though not extensive.

Vascular Changes. In all of the animals of the first group rather characteristic vascular changes occurred which we designate as "toxic vascular changes." These changes were widespread in all of the animals and occurred in both systemic and pulmonic circulations; small arteries, arterioles, capillaries, venules and small veins were most seriously affected. Toxic vascular changes consist of dilatation of the vessels; marked swelling to vacuolization of the endothelium with areas of endothelial proliferation; parenchymatous, vacuolar, or fatty degeneration of smooth muscle; hyaline swelling or rupture of elastic fibers; or areas of hyaline necrosis of portions of the entire thickness of the vascular wall. In many areas small vessels were actually ruptured, and increased permeability was repeatedly demonstrated by perivascular serous transudate and diapedesis of white and red blood cells.

Renal Glomerular Lesions. Associated with these vascular changes in all of the animals were definite acute glomerular lesions: swelling of glomerular endothelium, swelling and hyalinization of the glomerular epithelial basement membrane, some swelling of the epithelium, a consequent reduction of the blood content of the glomeruli, albuminuria, and occasional small glomerular hemorrhages. Rarely one to several polymorphonuclear leukocytes were found. These acute glomerular lesions gave no indication of proliferative reaction, which might have been expected had the animals survived for a longer time.

Lymphoid Tissue Changes. Most of these animals exhibited toxic and reactive lesions in the splenic corpuscles and lymph nodes examined. These consisted of some necrosis of lymphocytes, phagocytosis of lymphocytes, hyperplasia of germinal centers, and in some instances exhaustion and skeletonization of lymphoid tissue. Toxic vascular changes were prominent in these tissues.

Liver. No consistent lesions of the liver attributable to the administration of Dicumarol were discovered, and no necrosis of the liver was found in any of the 29 dogs. Some livers showed parenchymatous degeneration, some showed fatty degeneration, and a few hydropic degeneration. None of these degenerations was present in all of the animals, and there was no zonal distribution. In several instances sufficient time had elapsed post mortem or sufficient anemia resulting from hemorrhage had occurred to be an important consideration. Because of the nonspecificity of these degenerative lesions of the liver, their irregularity of appearance and of distribution, and the frequency with which all of them appear in dogs' livers under usual laboratory circumstances, it was felt that they could not be ascribed to Dicumarol, even with the large doses used. Of even greater significance was the lack of hepatic necrosis in the series.

Other Tissues. No other lesions appeared consistently. Occasionally a dog showed a purulent bronchiolitis or other secondary infection. Numerous minor lesions were seen, usually secondary to toxic vascular changes, but these were irregular or entirely incidental in occurrence. Fat stains, glycogen stains and Mallory's connective tissue stain were used frequently to confirm the findings.

Microscopic Findings in the Second Series

Upon the 6 dogs which received Dicumarol in dosages within therapeutic range over periods of from 1 to 2 months, autopsies were performed and sections of tissues comparable to those in the first were studied. The same special stains were used.

Particular attention was given to the lesions described as characteristic in the first series. Except in 1 dog, however, no gross or microscopic hemorrhages occurred. This animal (3A) suffered extensive hemorrhage about the neck after being injured in that region by another dog. In none of the tissues from these 6 dogs, including dog 3A, were there any toxic vascular changes. The renal glomerular changes were lacking in this group. On the other hand, 5 of these dogs showed varying degrees of toxic involvement of the lymphoid tissue of the spleen and lymph nodes.

In all 6 dogs, the only hepatic lesion found was a vacuolar appear-

ance. This vacuolization of liver cell cytoplasm was probably due to glycogen, and is believed to reflect a better state of nutrition in these animals kept on a good laboratory regime than in the dogs usually used in shorter experimental procedures. It is believed that this vacuolar change is not due to Dicumarol for the additional reason that it is accompanied by no evidence of toxic vascular change.

Discussion

The consistency of our observations of the pathology associated with large lethal and sublethal doses of Dicumarol in the first series of dogs would seem to indicate that they are significant. Most important of these are the definite evidences of serious damage to the small blood vessels. This damage, which is extensive in distribution, is apparently the result of specific toxicity of Dicumarol. Such pathologic changes in the vessel walls, combined with the induced reduction in prothrombin, make hemorrhages almost inevitable. That such hemorrhages, either gross or microscopic, do occur in Dicumarol poisoning is confirmed by our findings in this series of dogs.

That Dicumarol is toxic when given in large doses is further indicated by the consistent presence of toxic-reactive lymphoid tissue in the spleen, lymph nodes, lymph follicles and bone marrow. The hyperplastic reactive state of the lymphoid tissue indicates that in most instances this circulating toxin is not present in sufficient concentration to cause necrosis. And it also seems to indicate the probable site of detoxification in animals which survive.

The renal vascular changes are particularly noteworthy. These are most prominent and quite consistent in the glomeruli. The functional effects of this glomerular swelling were not studied in this series. But the morphologic changes suggest that the glomerular swelling may be of importance in various stages of acute poisoning, and there is a distinct possibility that significant secondary changes might have occurred had the animals lived longer.

We have failed to confirm other investigators' reports of liver necrosis or even to find consistent degenerative changes in the liver in Dicumarol poisoning in these dogs. We do not, therefore, agree that the lesions of primary importance are in the liver. On these morphologic grounds we question the theory that Dicumarol reduces prothrombin through destruction or injury of hepatic tissue.

The series of 6 dogs given Dicumarol in doses approximating those given human beings for therapeutic purposes contrast strongly with the larger series which were poisoned. Comparable effects on the prothrombin activity, as reflected by the prothrombin times, were seen

only in the dog that suffered trauma to the neck and died of extensive hemorrhages in that region. Yet in none of these animals were any of the lesions present which were seen in the previous series. Complete autopsies and extensive microscopic search failed to show toxic vascular change, hemorrhages, or renal glomerular swelling. However, some toxic tissue effect was noted in the hyperplastic reactive lymphoid tissue, but these changes were much less pronounced than in the previous series.

Human Cases

During, and since, the period when these animal experiments were being performed, autopsies have been conducted upon five human beings, with a variety of diseases, to whom Dicumarol had been administered. To these patients the drug was given in total doses of 250 to 5820 mg. over periods of several days to several weeks. Four of them received Dicumarol up to, or shortly before, death for varying periods of time; one had received a single dose of 305 mg. 2 days before death. The gross and microscopic findings have been reviewed with special reference to morphologic evidences of Dicumarol poisoning. None of the typical lesions has been found, nor have any other lesions which could be unequivocally charged to Dicumarol. No hepatic necrosis or degeneration ascribable to Dicumarol was determined, and no secondary or chronic lesions due to Dicumarol. Hyperplastic lymphoid reaction was seen in several instances, but other changes obscured the background.

Summary

In a series of 29 dogs which had received doses of Dicumarol far larger than necessary or desirable for therapeutic purposes, the outstanding morphologic evidences of poisoning were:

1. Hemorrhages, gross or microscopic
2. Toxic lesions of small vessels
3. Acute renal glomerular swelling
4. Toxic lymphoid tissue reaction

No necrosis of the liver and no consistent hepatic degenerative lesions were found.

In a series of 6 dogs which had received Dicumarol at levels of therapeutic dosage, the first three lesions listed were not seen, and the lymphoid tissue reaction was milder.

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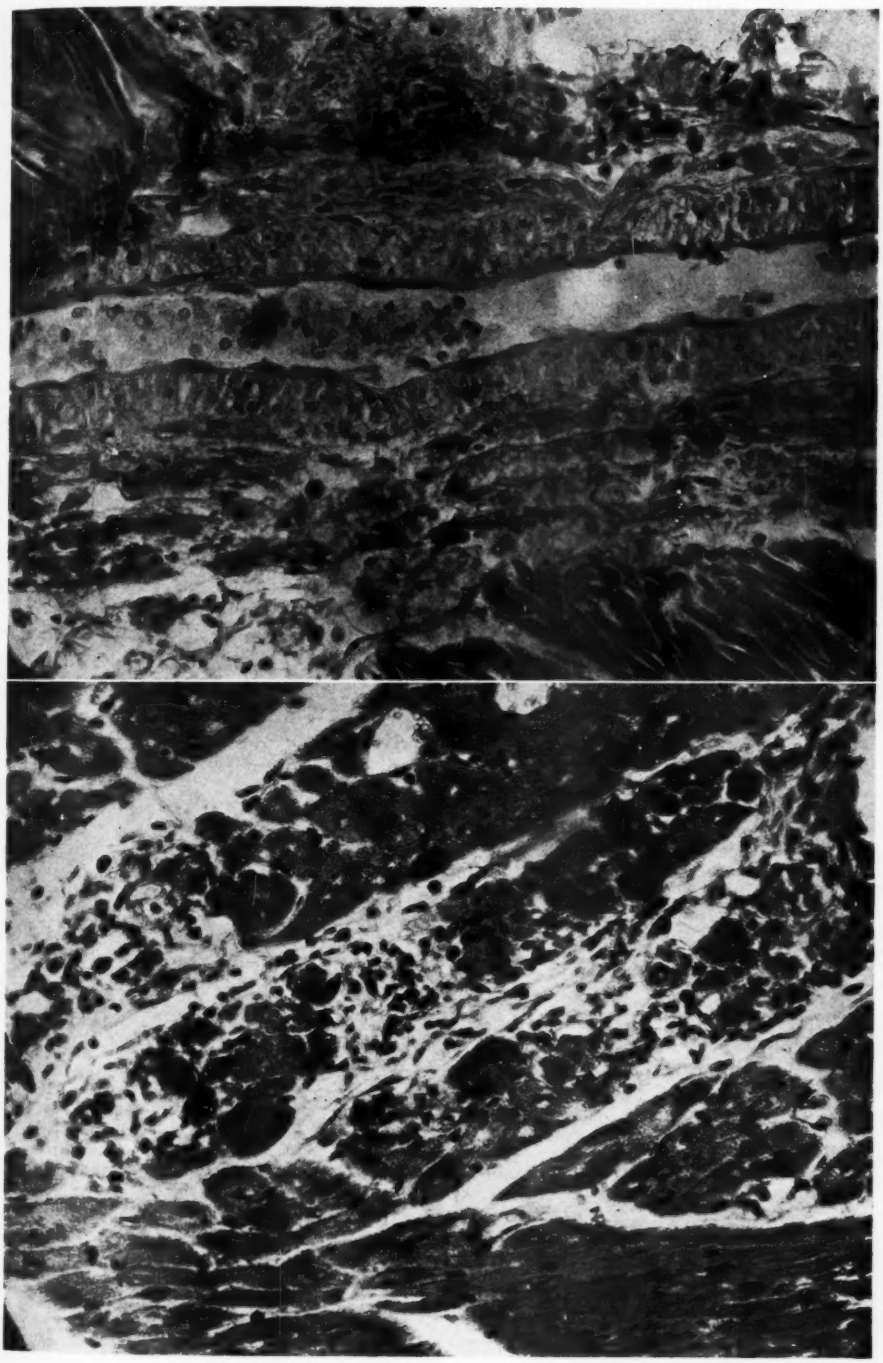
[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 130

FIG. 1. Small artery in the wall of the urinary bladder of a dog poisoned with Dicumarol, showing endothelial swelling, vacuolation of smooth muscle, infiltration of vessel wall by inflammatory cells, and surrounding tissue edema. × 400.

FIG. 2. Myocardium of a dog poisoned with Dicumarol, showing endothelial swelling, capillary rupture, toxic necrosis of adjacent muscle fibers, and early inflammatory reaction. × 400.



McCarter, Bingham and Meyer Pathologic Findings After Dicumarol

EXTRACTS FROM MINUTES OF THE MEETING OF THE COUNCIL

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CLEVELAND

APRIL EIGHTH, 1944

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Extracts from Minutes of the Meeting of the Council Held at Cleveland, Ohio, April 8, 1944

Present. President CANNON, Doctors FORBUS, HAYTHORN, KARSNER, MORITZ, SOULE and WELLER.

The Secretary reported that leave of absence because of military service has been granted to the following: Drs. Leo Alexander, D. Murray Angevine, William Antopol, Francis Bayless, Eustace L. Benjamin, Louis F. Bishop, Jr., Herman Bolker, Mark M. Bracken, Chester R. Brown, John W. Budd, Warren C. Corwin, Milton J. Eisen, W. Norman Elton, George H. Fetterman, Edgar C. Fielden, Harold Fink, James S. Forrester, Edward A. Gall, R. H. Goodale, Thomas R. Hamilton, Francis F. Harrison, J. Beach Hazard, Henry Horn, Cornelius A. Hospers, George J. Kastlin, Harold B. Kenton, Jack D. Kirshbaum, Leone McGregor, Abou D. Pollack, Clara Raven, Sol R. Rosenthal, Joseph E. Smadel, William S. Stanbury, M. J. Stewart, Phillips Thygeson, H. Windsor Wade, Robert J. Williams, Harold Wood.

The following were elected to membership in the Association:

LAWRENCE BERMAN
WILBERT OTHO BROWN
ALFONS PHILIP FALKENSTEIN
ROBERT A. FOX
NATHAN B. FRIEDMAN
JOHN E. GREGORY
JOSEPH MACGLASHAN HILL
HARALD N. JOHNSON
EMILY NICHOLS LOEB
ELIZABETH LOWENHAUPT
GEORGE MARGOLIS
EDMUND MAYER
G. BURROUGHS MIDER
HARRY E. MORTON
BALBIR BALL GREENE NEHAUL
HARRY GEORGE OLKEN
OTAKAR J. POLLAK
JOSEPH-LUC RIOPELLE
WILLIAM OGBURN RUSSELL
BERT E. STOFER
ROBERT E. STOWELL
GEORGE SIEGISMUND STRASSMANN
WALTER ALBERT STRYKER
FRANK THORP, JR.
JOHN LINCOLN WORK

It was voted to require payment of first year's dues of new members in order to establish membership, with understanding that the *American Journal of Pathology* will be sent for the entire year 1944, and with the

further understanding that those in military service may apply for leave of absence to begin January 1, 1945.

At their request, Drs. Percival Bailey and W. Bloom were reinstated to membership.

The deaths of the following members were recorded with deep regret:

Frank C. Andrus	Frederic C. Narr
Carl W. Apfelbach	G. Y. Rusk
Leo Buerger	A. V. St. George
George W. Crile	W. R. Steiner
James Ewing	Leslie T. Webster
Karl Lowenthal	H. G. Wells

Dr. Malcolm H. Soule was reelected Associate Editor of the *American Journal of Pathology* for a term of one year.

Dr. Paul R. Cannon, whose term expires as a member of the Editorial Board of the *American Journal of Pathology,* was reelected for a term of six years.

It was voted that unless unforeseen circumstances arise, the annual scientific sessions of the Association will be held at the University of Chicago, Friday and Saturday, May 4 and 5, 1945. The symposium selected for the meeting is "Infectious Granulomas, Exclusive of Tuberculosis and Syphilis." Dr. Wiley D. Forbus, Professor of Pathology of Duke University, will be the referee.

The Council voted unanimously to select Dr. Ludvig Hektoen for the award of the Gold Headed Cane.

Dr. N. Chandler Foot was appointed as Chairman of the Advisory Committee of the Lymphatic Tumor Registry.

It was voted to appoint Dr. Harry Goldblatt as a new member of the Advisory Committee of the Lymphatic Tumor Registry.

It was voted that the Council of the American Association of Pathologists and Bacteriologists recommend to the Council of the International Association of Medical Museums consideration of a discussion of pedagogical and educational problems in the field of Pathology.

Howard T. Karsner, *Secretary*